



US009422450B2

(12) **United States Patent**
Irvin et al.(10) **Patent No.:** **US 9,422,450 B2**(45) **Date of Patent:** ***Aug. 23, 2016**(54) **SURFACE COATED STRUCTURES AND METHODS**(75) Inventors: **Randall Thomas Irvin**, Sherwood Park (CA); **Elisabeth Melika Davis**, Edmonton (CA); **Dongyang Li**, Edmonton (CA); **Daniel Abraham Muruve**, Calgary (CA)(73) Assignee: **ARCH BIOPHYSICS, INC.**, Sherwood Park (CA)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 159 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **14/111,701**(22) PCT Filed: **Apr. 13, 2012**(86) PCT No.: **PCT/CA2012/000360**

§ 371 (c)(1),

(2), (4) Date: **Apr. 4, 2014**(87) PCT Pub. No.: **WO2012/139208**PCT Pub. Date: **Oct. 18, 2012**(65) **Prior Publication Data**

US 2014/0220368 A1 Aug. 7, 2014

Related U.S. Application Data

(63) Continuation of application No. 13/087,257, filed on Apr. 14, 2011, now Pat. No. 9,096,775, which is a continuation-in-part of application No. 12/889,958, filed on Sep. 24, 2010, now Pat. No. 7,998,359.

(60) Provisional application No. 61/249,964, filed on Oct. 8, 2009.

(51) **Int. Cl.**

A61K 39/00	(2006.01)
A61K 39/108	(2006.01)
A61K 39/02	(2006.01)
C09D 179/00	(2006.01)
A01N 37/46	(2006.01)
A01N 63/02	(2006.01)
A61B 5/145	(2006.01)
A61L 27/04	(2006.01)
A61L 27/22	(2006.01)
A61L 27/34	(2006.01)
A61L 27/54	(2006.01)
C07K 14/21	(2006.01)

(52) **U.S. Cl.**CPC **C09D 179/00** (2013.01); **A01N 37/46** (2013.01); **A01N 63/02** (2013.01); **A61B 5/14532** (2013.01); **A61B 5/14546** (2013.01); **A61L 27/04** (2013.01); **A61L 27/227** (2013.01); **A61L 27/34** (2013.01); **A61L 27/54** (2013.01); **C07K 14/21** (2013.01); **A61L 2300/25** (2013.01); **A61L 2400/18** (2013.01); **Y10T 428/31725** (2015.04)(58) **Field of Classification Search**CPC **A61K 39/00**; **A61K 39/02**; **A61K 39/104**
USPC **424/184.1**, **234.1**, **260.1**
See application file for complete search history.(56) **References Cited****U.S. PATENT DOCUMENTS**

9,096,775 B2 * 8/2015 Irvin et al.

FOREIGN PATENT DOCUMENTS

WO WO 2007/089272 A 8/2007

OTHER PUBLICATIONSGiltner et al., "The pseudomonas aeruginosa type IV pilin receptor binding domain functions as an adhesion for both biotic and abiotic surfaces", *Molecular Microbiology*, vol. 59, No. 4, pp. 1083-1096 (2006).

International Search Report from related PCT Patent Application No. PCT/CA2010/001612 mailed on Jan. 19, 2011, Application now published as International Publication No. WO 2011/041906 on Apr. 14, 2011.

Lombardo et al., "Initial studies of protein nanotubule oligomerization from a modified gold surface", *J. Bionanoscience*, vol. 3, No. 1, pp. 61-65 (2009).Yu et al., "Surface nanocrystallization of stainless steel for reduced biofilm adherence", *Nanotechnology*, vol. 19, pp. 1-8 (2008).

* cited by examiner

Primary Examiner — Rodney P Swartz(74) *Attorney, Agent, or Firm* — King & Spalding LLP(57) **ABSTRACT**

The present invention relates to a methods and composition in which a D-form or retro-inverso *Pseudomonas* pilin peptide, optionally having a polyethylene glycol moiety covalently attached to the peptide, is bound to a substrate. The disclosed methods and composition are useful for reducing the frictional coefficient, inhibiting biofilm formation by non-*Pseudomonas* bacteria, for reducing the inflammatory response to a material, for reducing corrosion of metals, and in biosensor applications.

19 Claims, 33 Drawing Sheets

G7-G8 SIDWGCASDSNAVS SSGTDRNMPALTAGTLFARFAPSECR SEQ ID NO:1
 PA110594 TIDWACTSASNAT--ATAQGFTGMAAGSVPQEFAPAQCR SEQ ID NO:2
 PAQ DGVWACKSTQDPMFTPKGCDN SEQ ID NO:3
 PAK DGLWKCTSDQDEGFIPKGCSE SEQ ID NO:4
 PA1244 DGVWNCKITKTPTAWKPNYAPANCPKS SEQ ID NO:5
 PA5668 EGVWTCATSGSPANWKANYAPANCPKS SEQ ID NO:6
 PA82935 NGGWSCATTVDKFRPNGCTD SEQ ID NO:7
 PA5198 GSSWACGNASIDGFAGTGTTIDAKYLPNACKP SEQ ID NO:8
 K122-4 SYTWACTSNADNKYLPKTCQTATTTTP SEQ ID NO:9
 Composite: K/A/S/T-C-T/K/A-S/T-D/T/N-Q/V/A-D/E-E/P/A/N-Q/M/K-F/Y-Y/T/R/L-P-K/N-G/T-C-S/D/T/Q/N-
 K/N/D/T (SEQ ID NO:10).
 Composite: S/T-I-D-W-G/A-C-A/T-S-D/A-S-N-A-V/T-S/-S-G/A-T-D/A-R/Q-W/G-M/F-P/T-A/G-L/M-T/A-A-G-
 T/S-L/V-P-A/Q-R/E-F-A-P-S/A-E/G-C/R (SEQ ID NO:21)

Fig. 1A

K122-4 SYTWACT S----NADNKYLPKTCQT-----TTTTT SEQ ID NO:11
 Rmet2278954 SVTWQCE S----SADKRYVPOACAKASESGKTTTTTTT SEQ ID NO:12
 Ncin7259488 SFSWYCK KGTSGGVDDKFLPSSCRTA-----ATTTAG SEQ ID NO:13
 Ecor729393 SFSWECSS S----NADAKYLPSSCRNA-----ATPTPT SEQ ID NO:14

Fig. 1B

PA5198 GSSWACGNASIDG-----FAGTGTIDAKYLPNACKP----- SEQ ID NO:15
 Ecor729393 SFSWECSS-----NADAKYLPSSCRNAATPTPTPT SEQ ID NO:16
 Ncin95402 SVKWFCGQPVTRTGDNDDTVADAKDGKEIDTKHLPSTCRDTSSAGK- SEQ ID NO:17
 Xcam21232529 SISWGCTN-----GTTIDGKYLPTSCRTAAAP---- SEQ ID NO:18

Fig. 1C

PA82935 NGGWSCATTVDKFRPNGCTD---- SEQ ID NO: 19
 Dnod120452 SSSWYCHGNAAEKFLPSGCKYDASL SEQ ID NO: 20
 PA82935 NGGWSCATTVDKFRPNGCTD---- SEQ ID NO: 19
 Dnod120452 SSSWYCHGNAAEKFLPSGCKYDASL SEQ ID NO: 20

Fig. 1D

Rmet = *Ralstonia metallidurans* GI 2278954
 Ncin = *Neisseria cinerea* GI 7259488
 Ecor = *Escherichia coli* GI 729393
 Ncin = *Neisseria gonorrhoeae* GI 95402
 Xcam = *Xanthomonas campestris* GI 21232529
 Dnod = *Dichelobacter nodosus* GI 120452

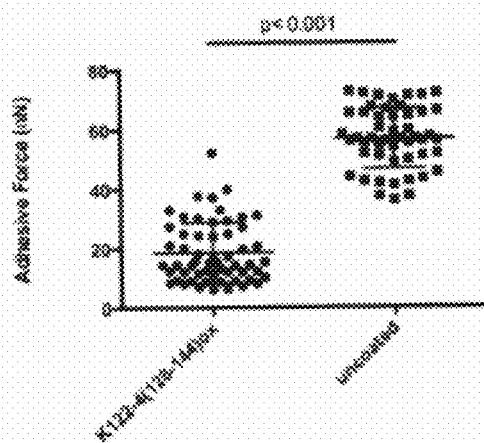


Fig. 2A

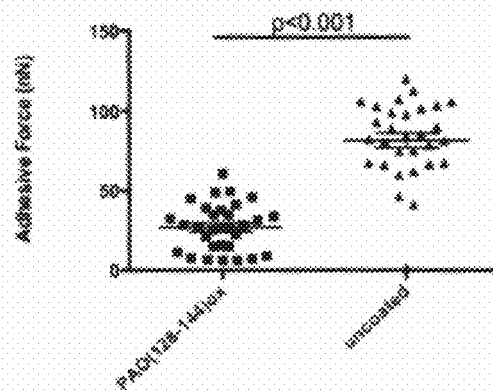


Fig. 2B

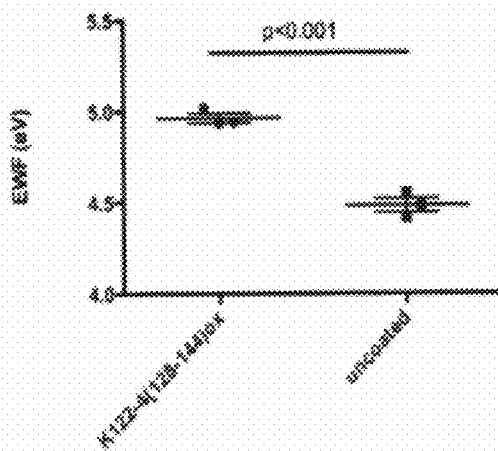


Fig. 3A

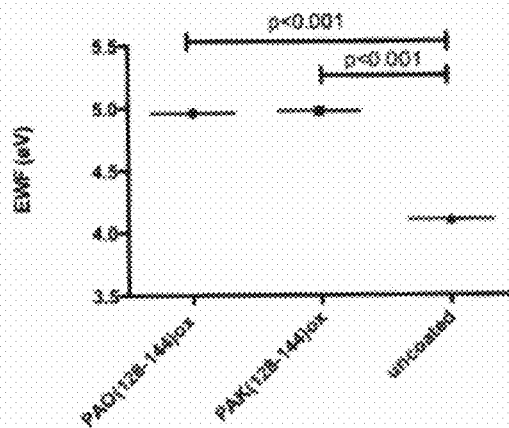


Fig. 3B

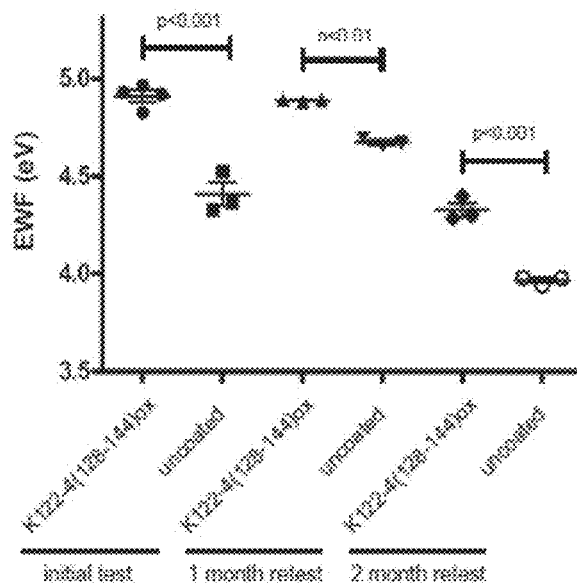


Fig. 4

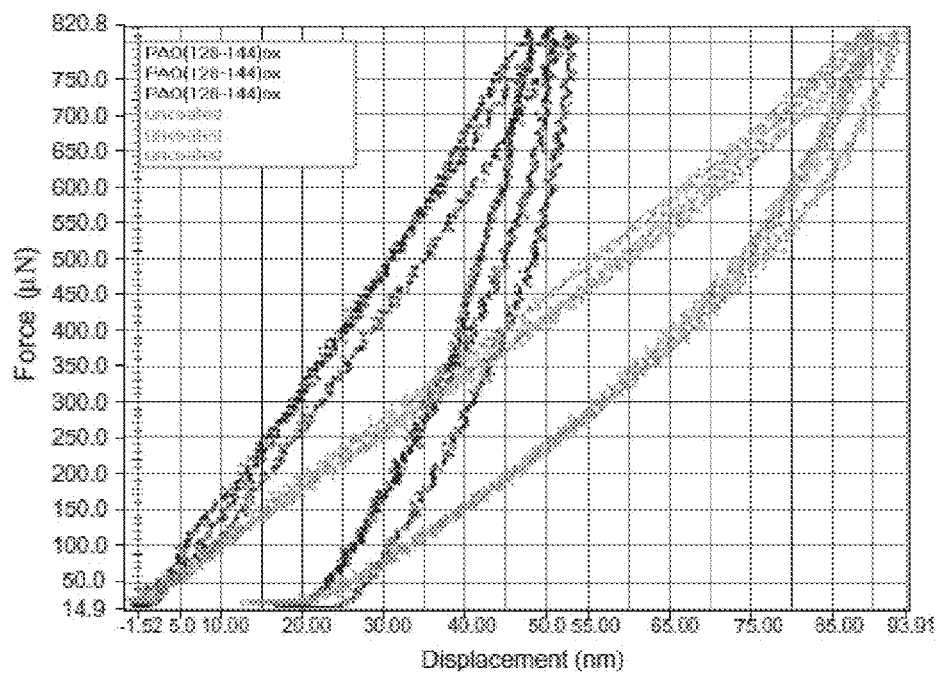


Fig. 5

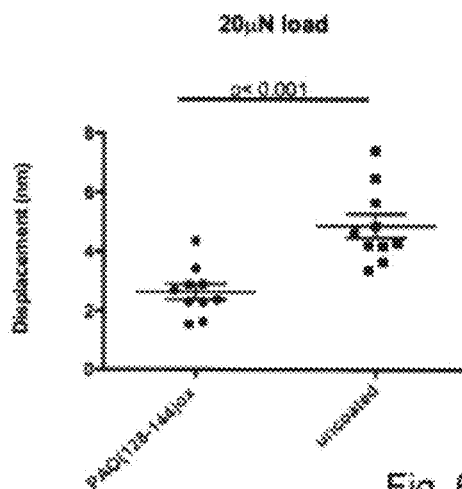


Fig. 6A

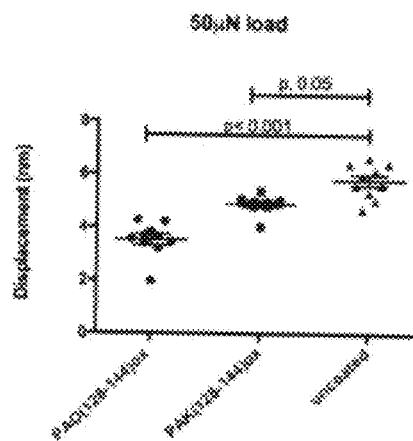


Fig. 6B

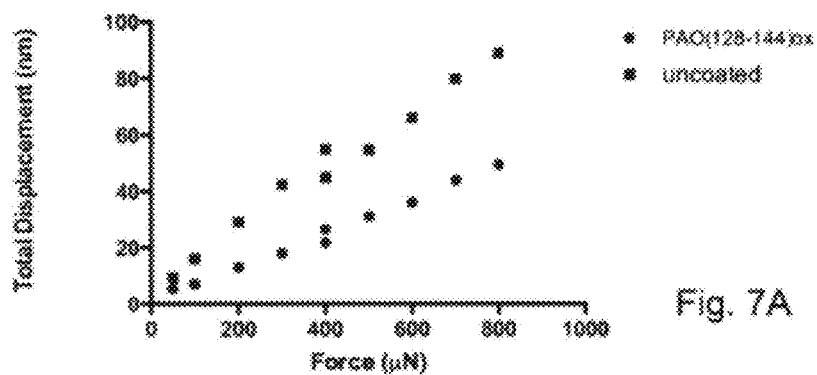


Fig. 7A

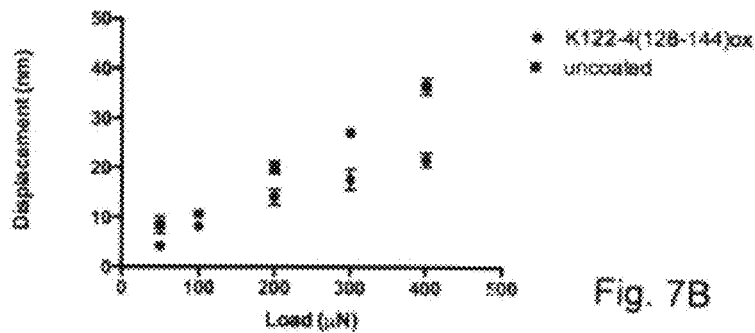


Fig. 7B

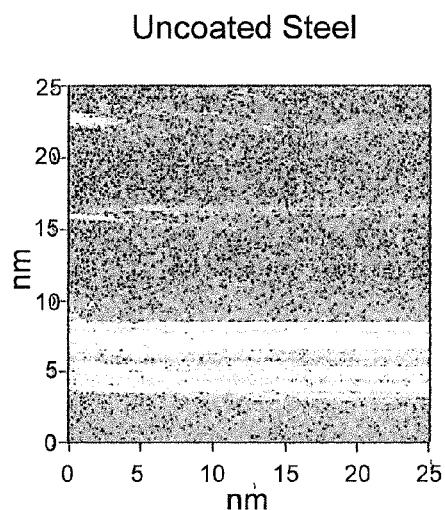


Fig. 8A

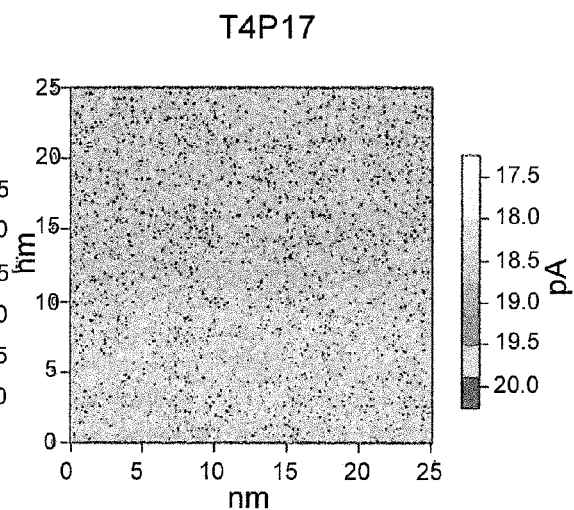


Fig. 8B

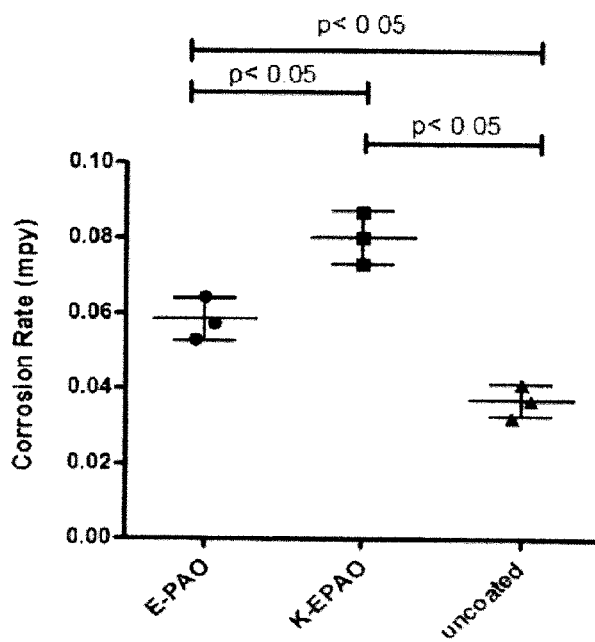


Fig. 11

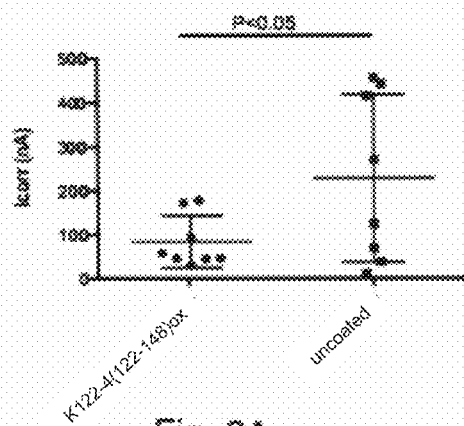


Fig. 9A

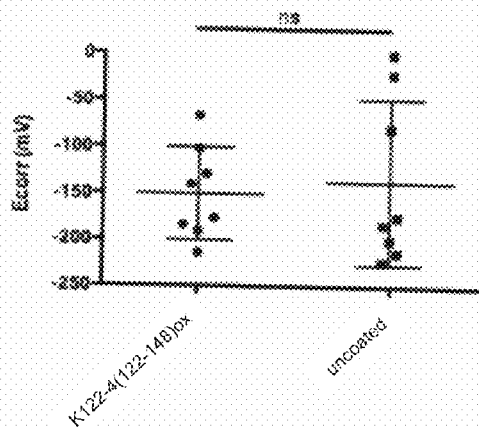


Fig. 9B

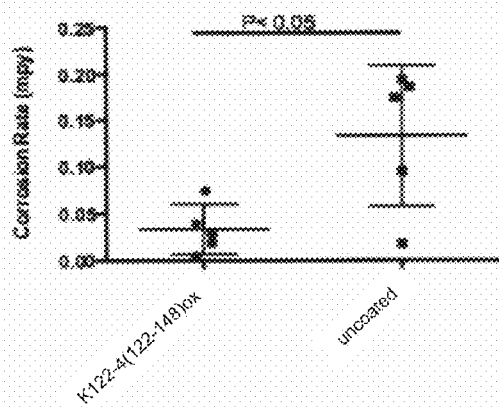


Fig. 10A

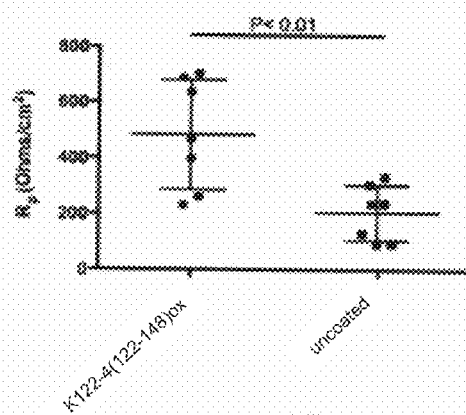


Fig. 10B

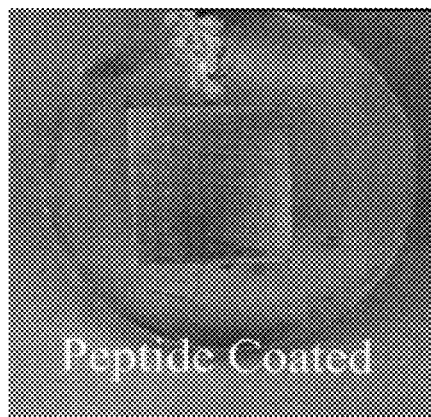


Fig. 12A

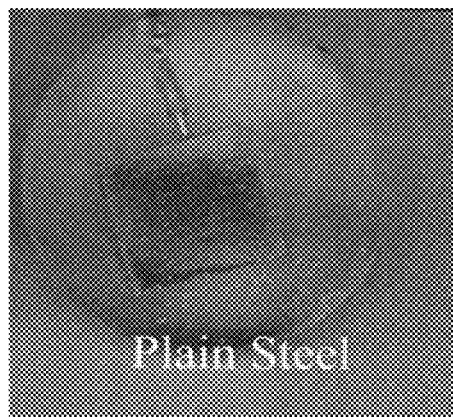


Fig. 12B

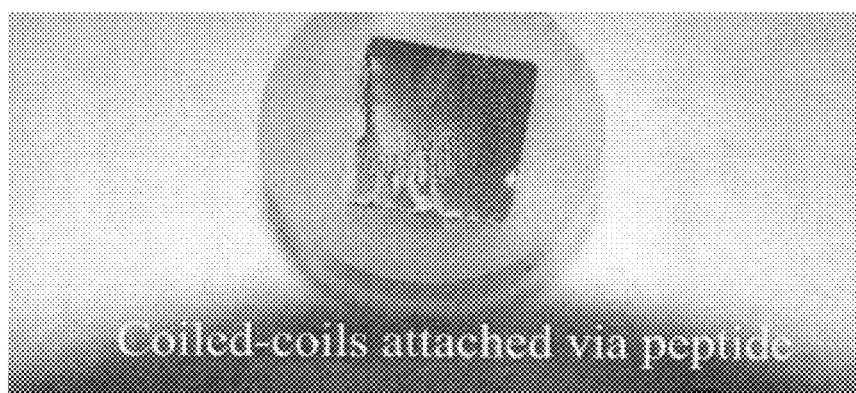


Fig. 12C

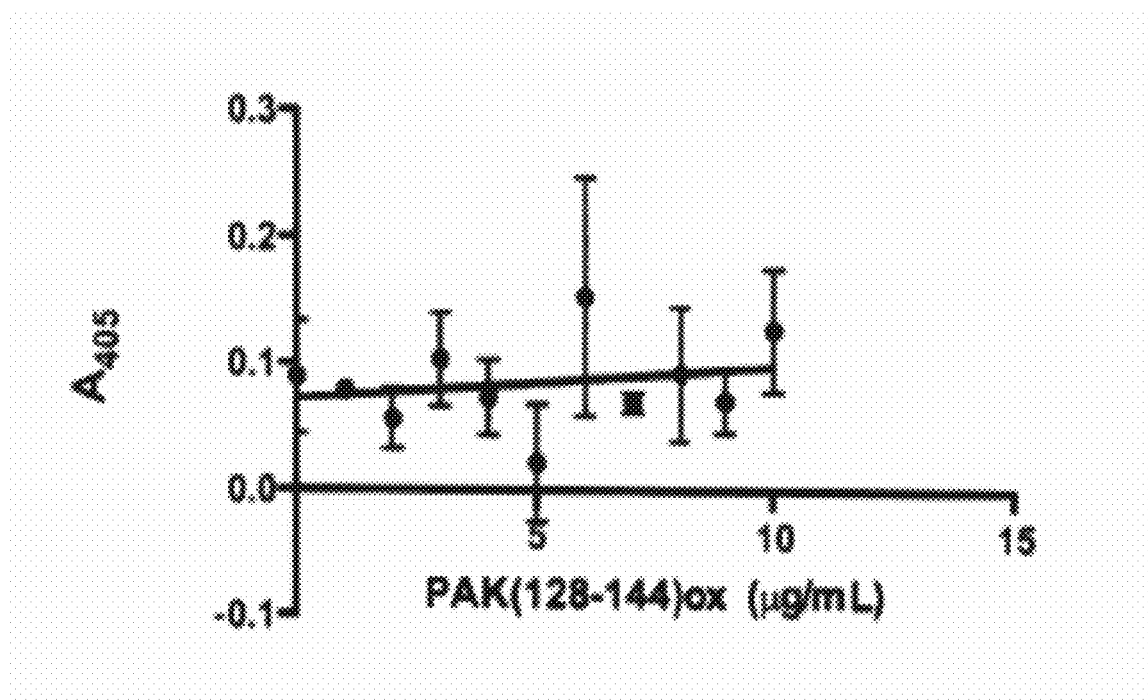


Fig. 13

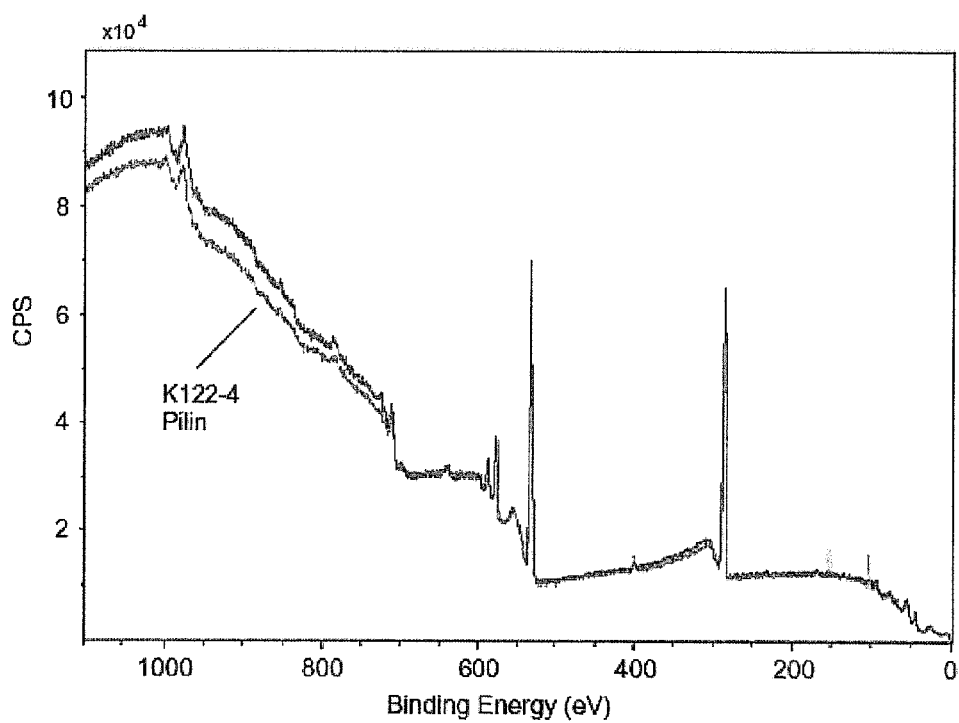


Fig. 14

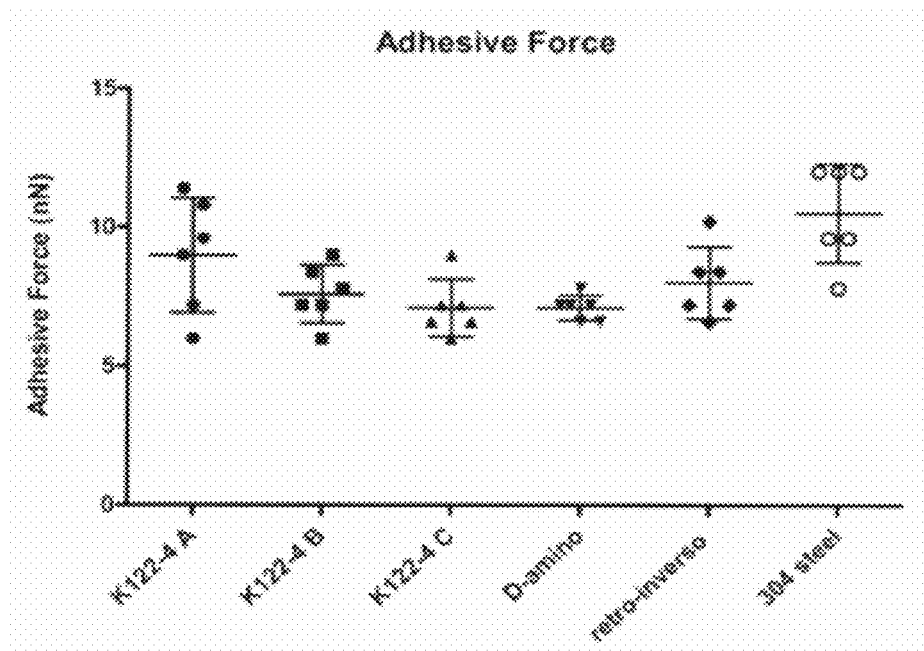


Fig. 15A

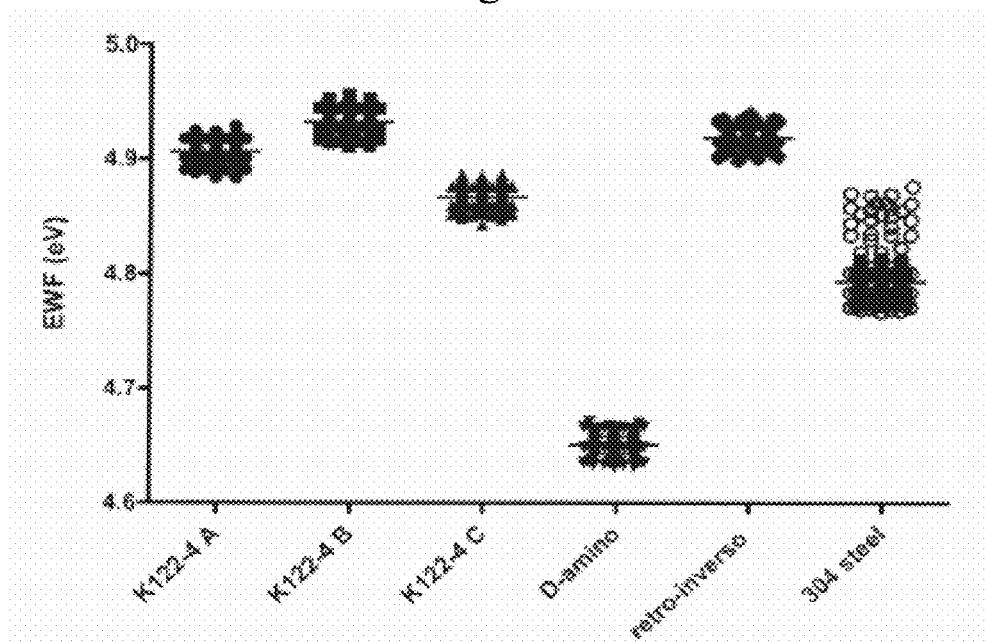


Fig. 15B

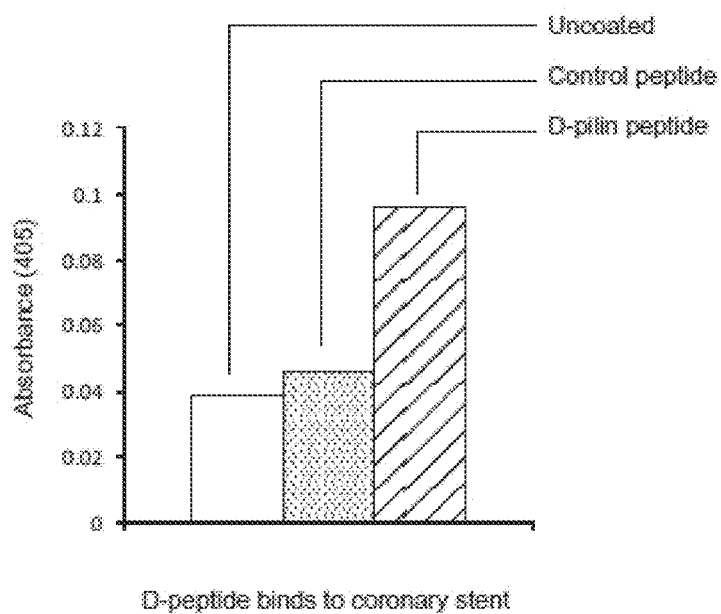


Fig. 16A

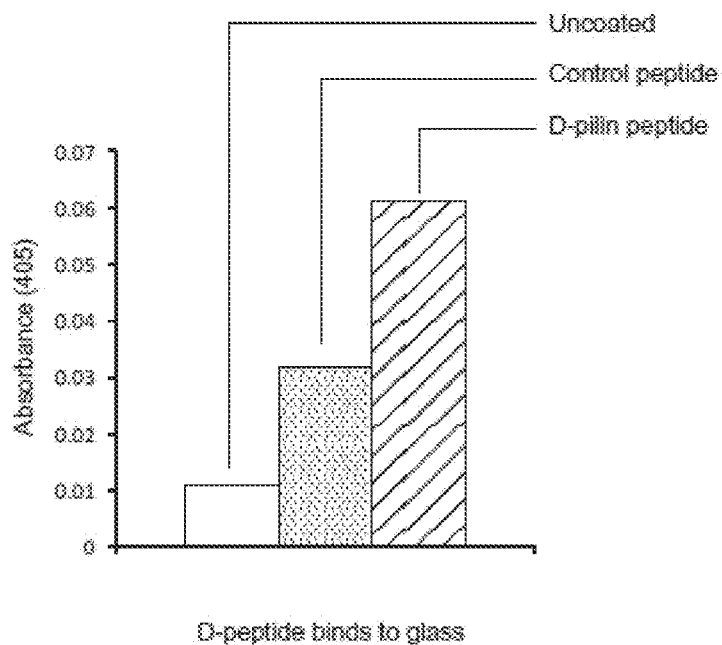


Fig. 16B

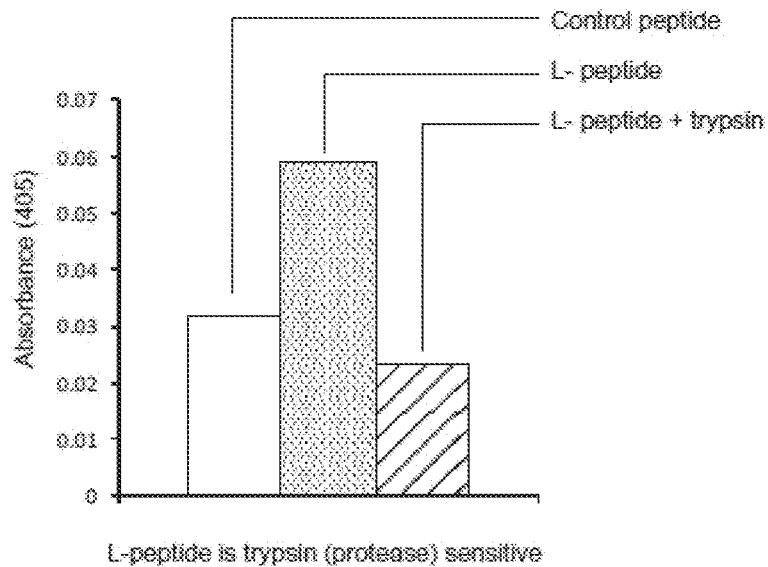


Fig. 17A

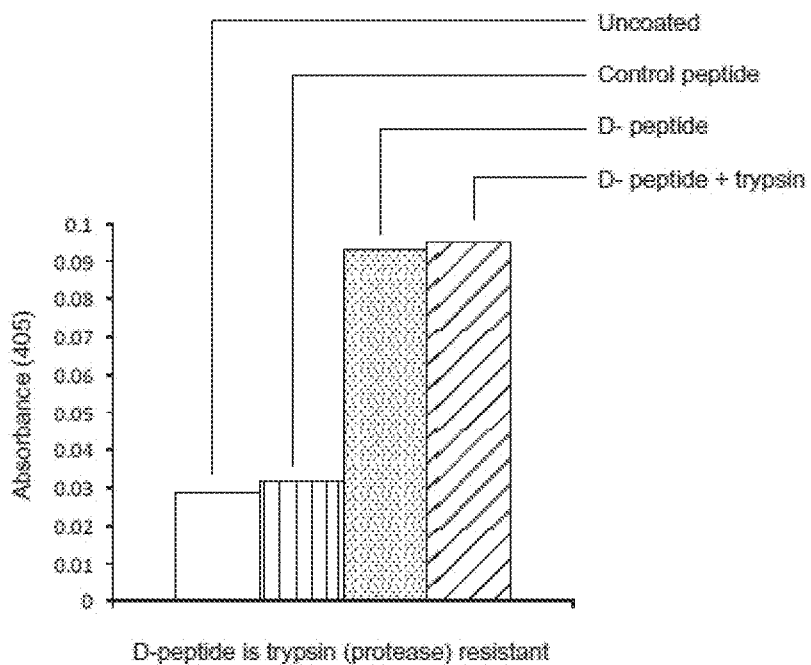


Fig. 17B

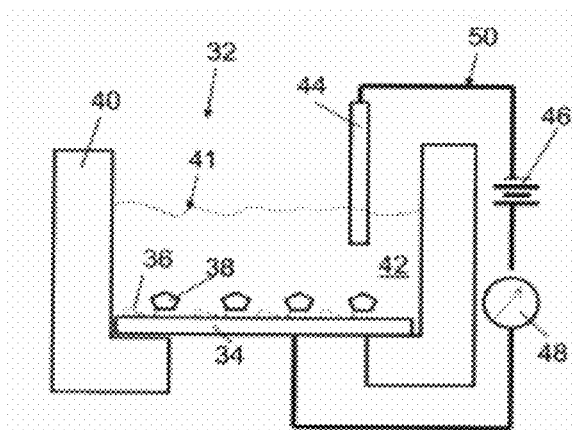


Fig. 18A

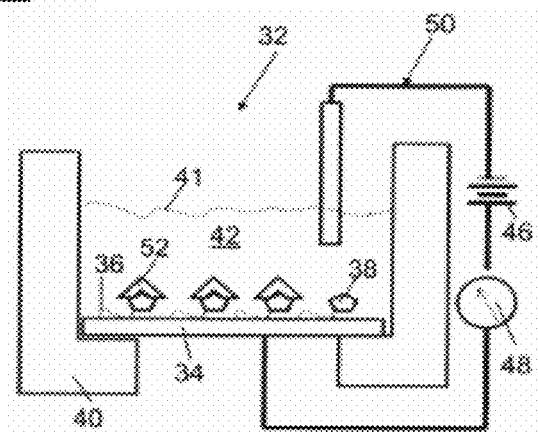


Fig. 18B

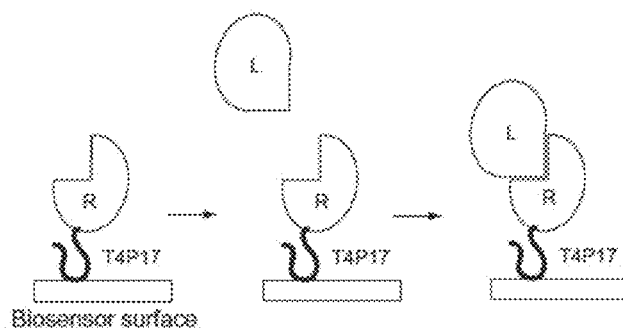


Fig. 19

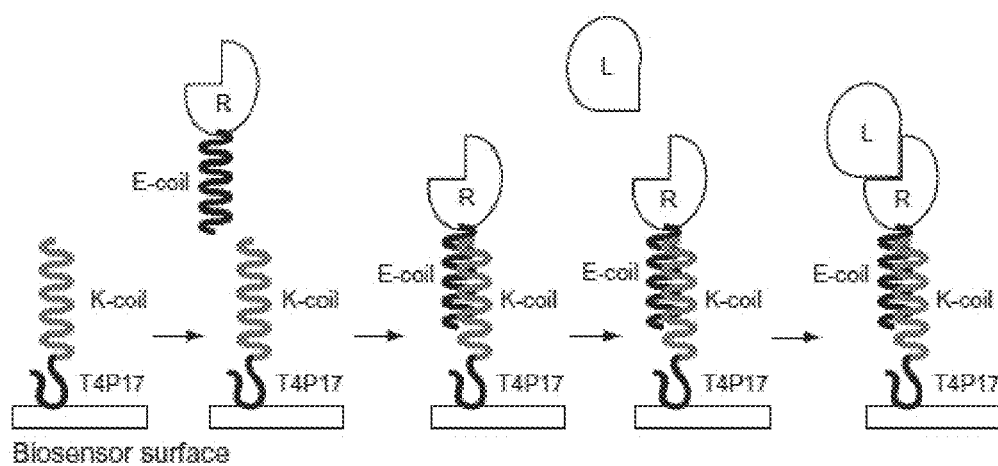


Fig. 20

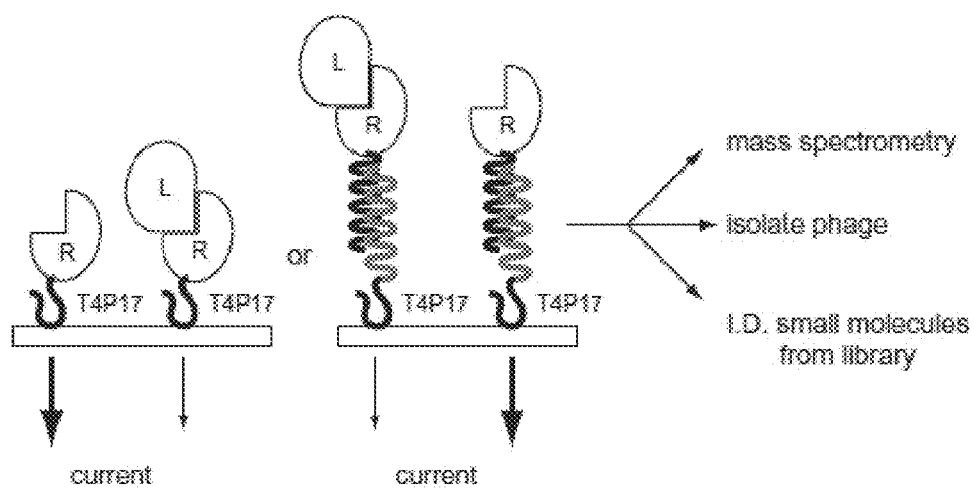


Fig. 21

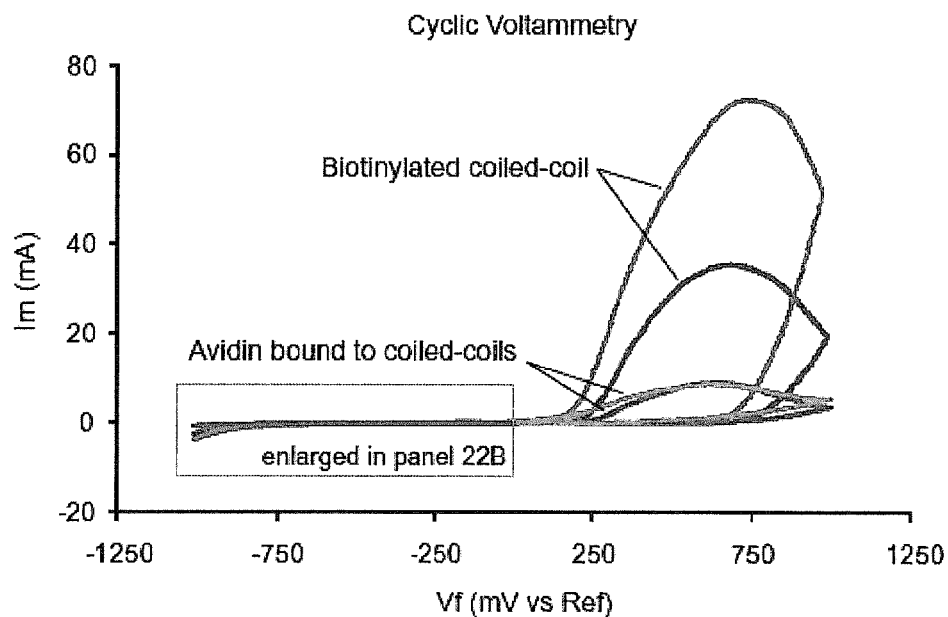


Fig. 22A

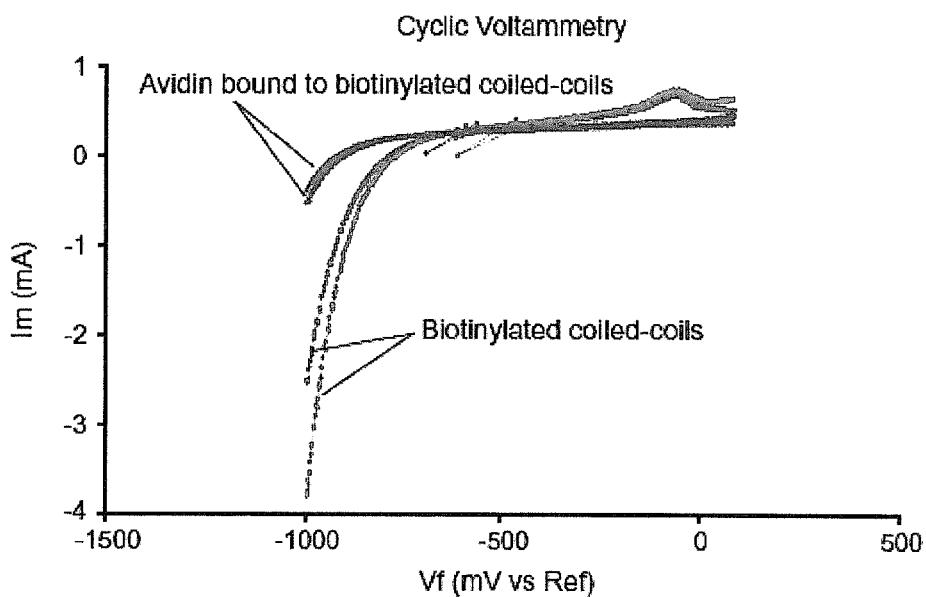


Fig. 22B

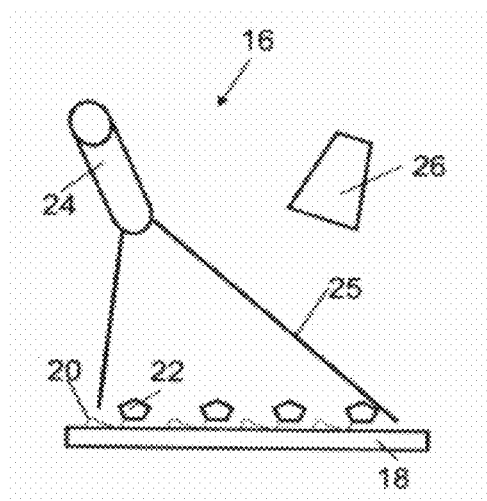


Fig. 23A

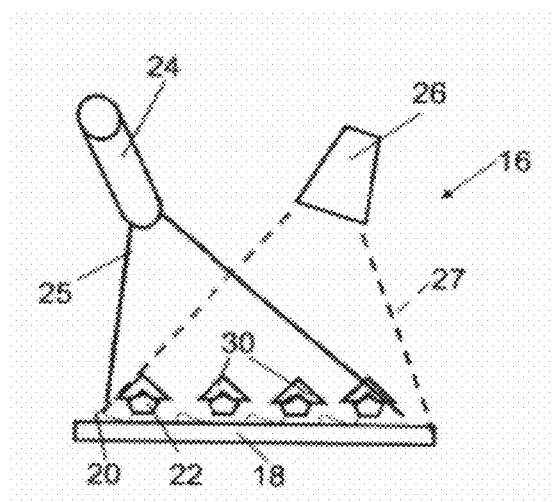


Fig. 23B

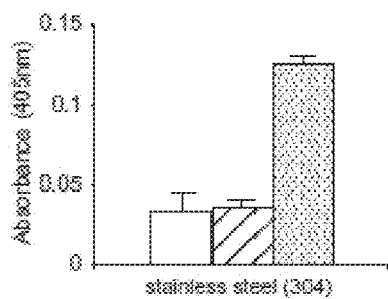


Fig. 24A

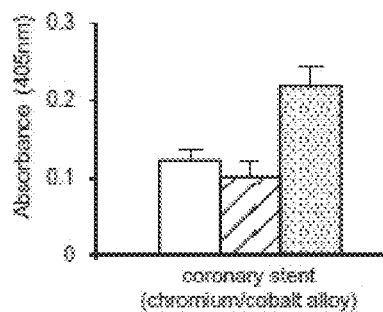


Fig. 24B

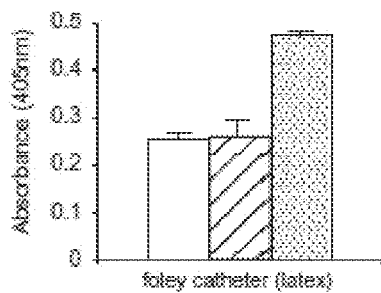


Fig. 24C

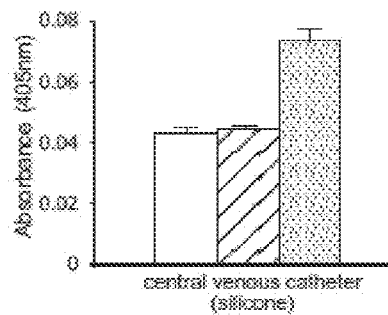


Fig. 24D

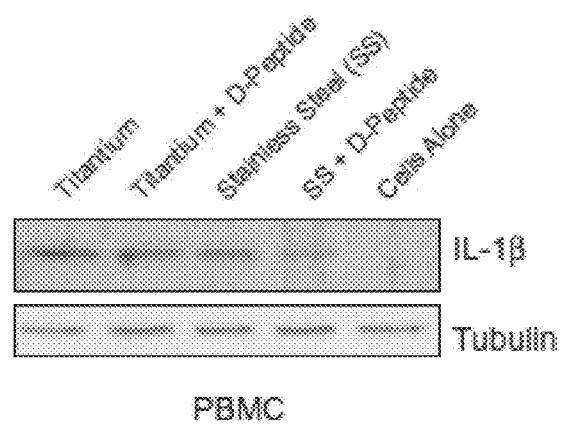


Fig. 25A

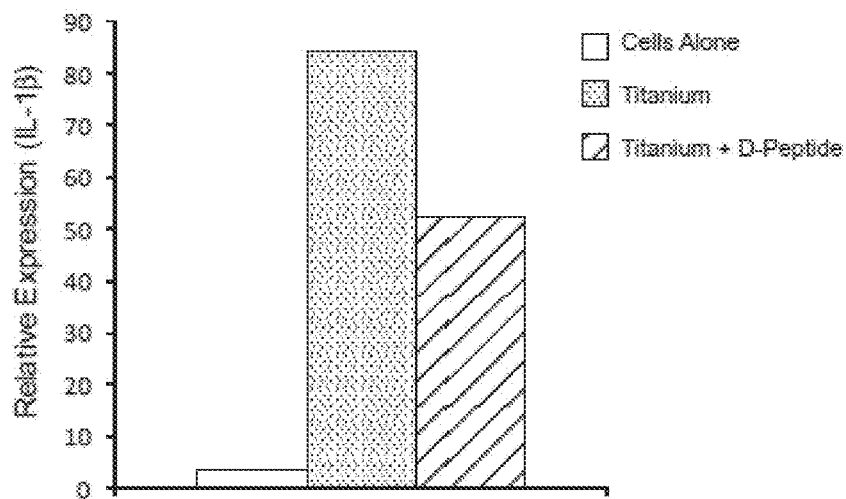


Fig. 25B

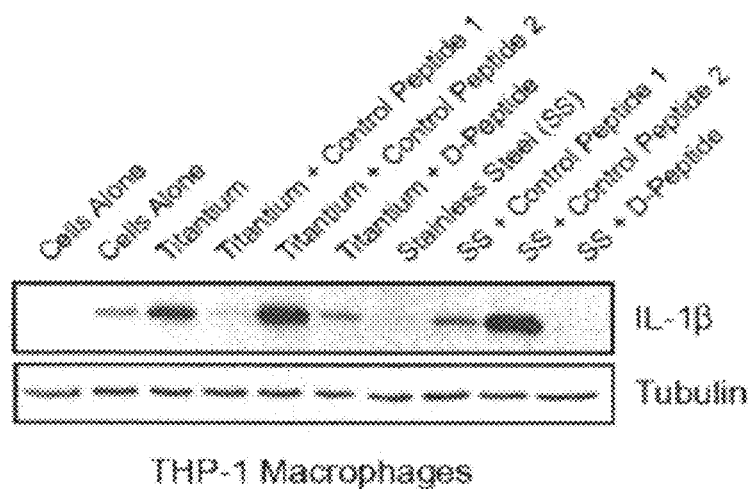


Fig. 26A

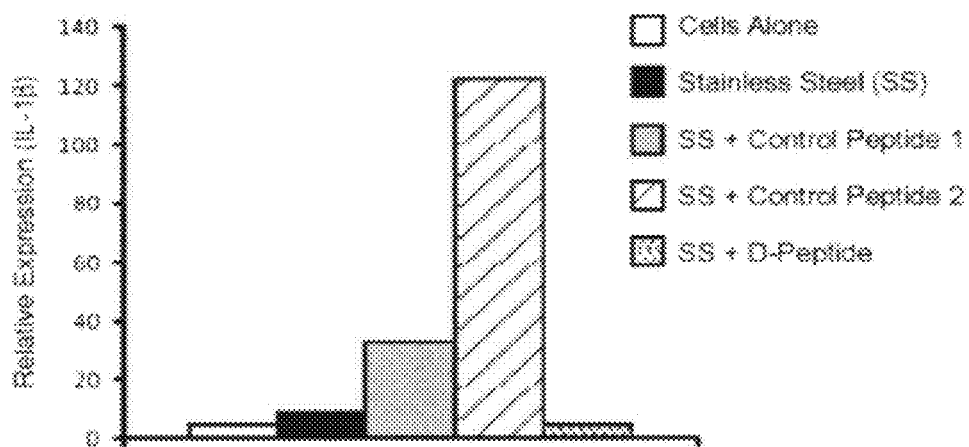


Fig. 26B

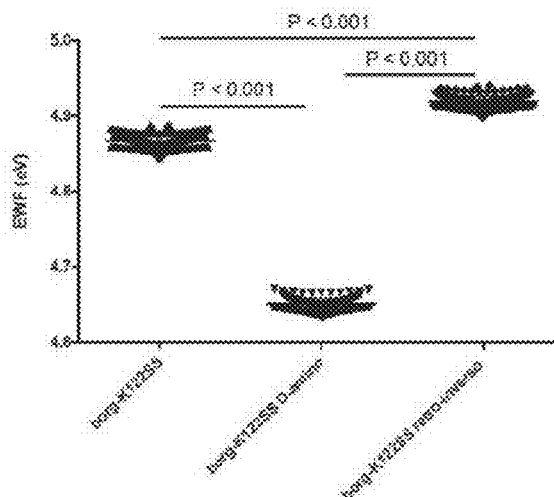


Fig. 27A

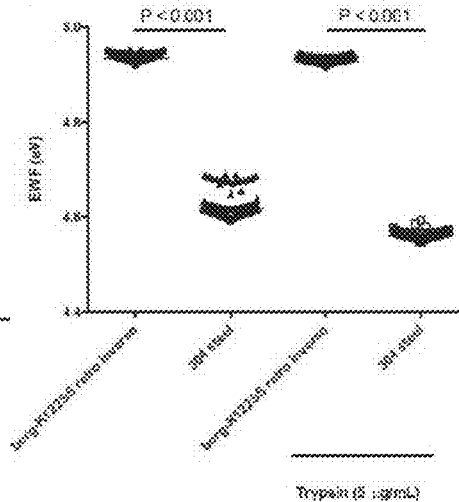


Fig. 27B

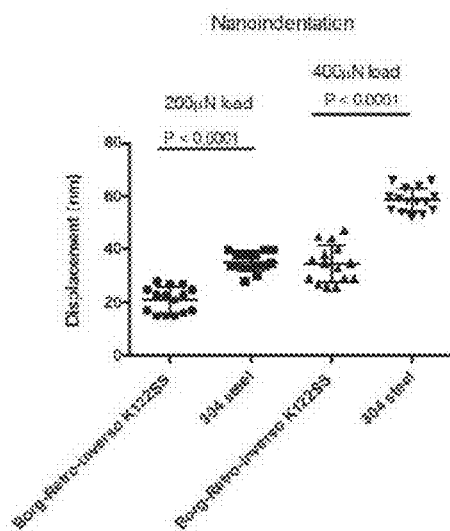


Fig. 27C

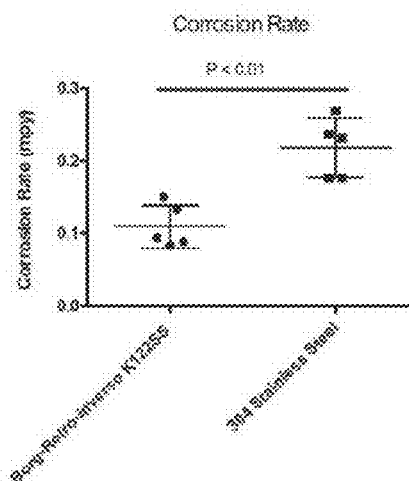


Fig. 27D

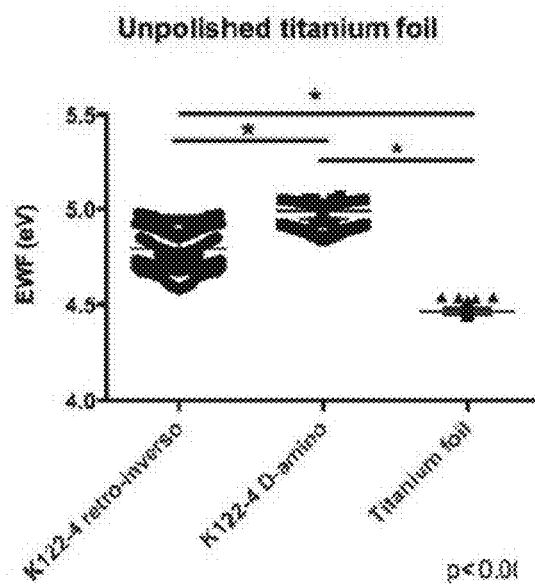


FIG. 28A

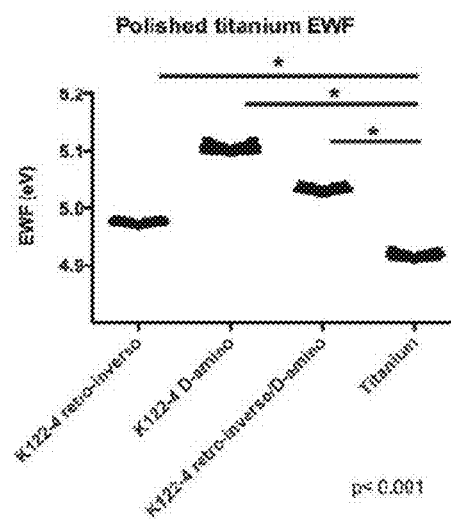


FIG. 28B

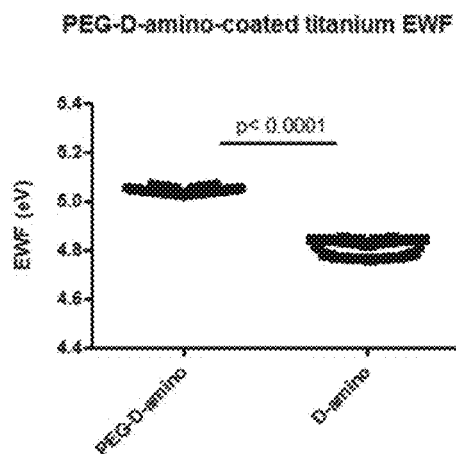
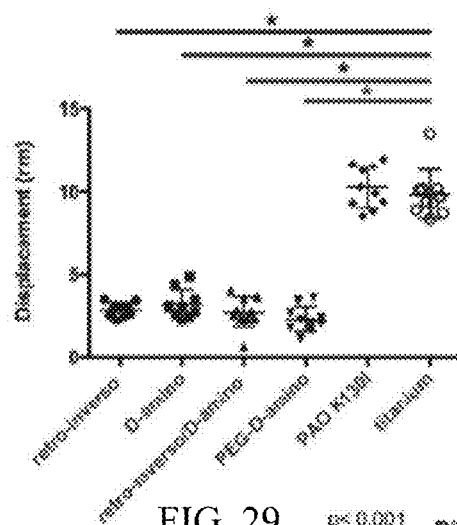


FIG. 28C

Titanium nanoindentation with 1mN peak load



Displacement at 1mN with final force of 100mN

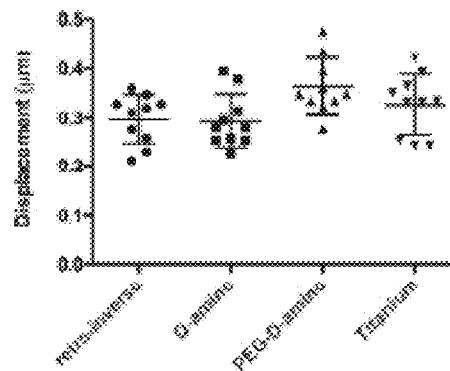


FIG. 30A

Displacement at 10mN with final force of 100mN

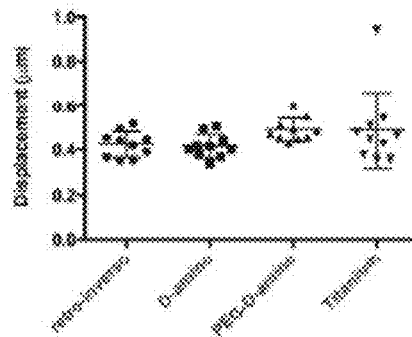


FIG. 30B

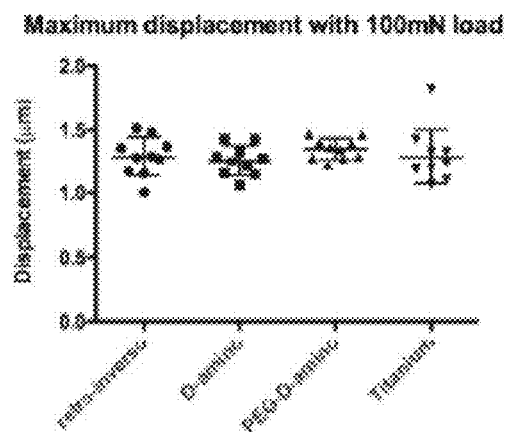


FIG. 30C

Displacement at 10mN force using a final force of 300mN

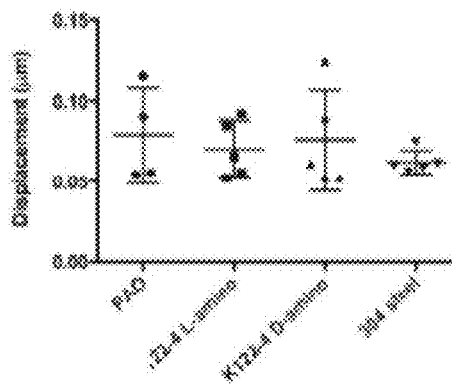


FIG. 30D

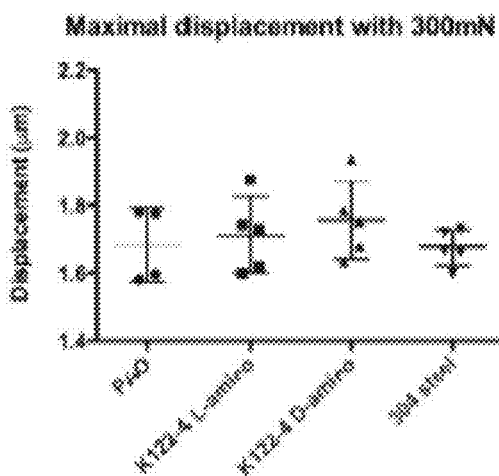


FIG. 30E

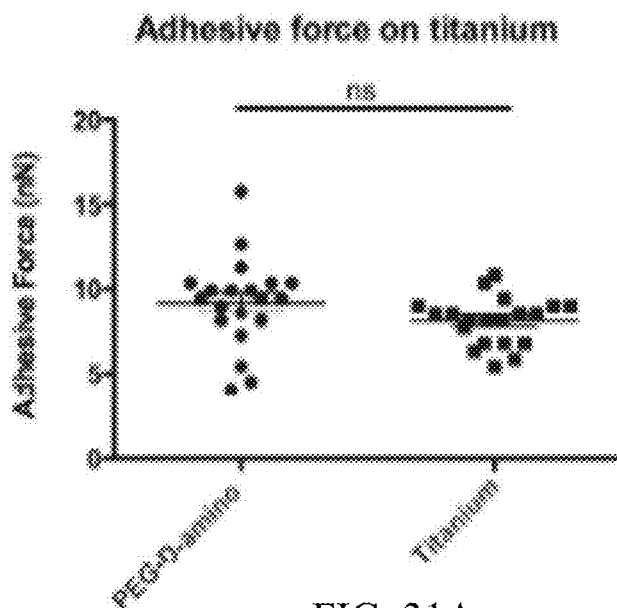


FIG. 31A

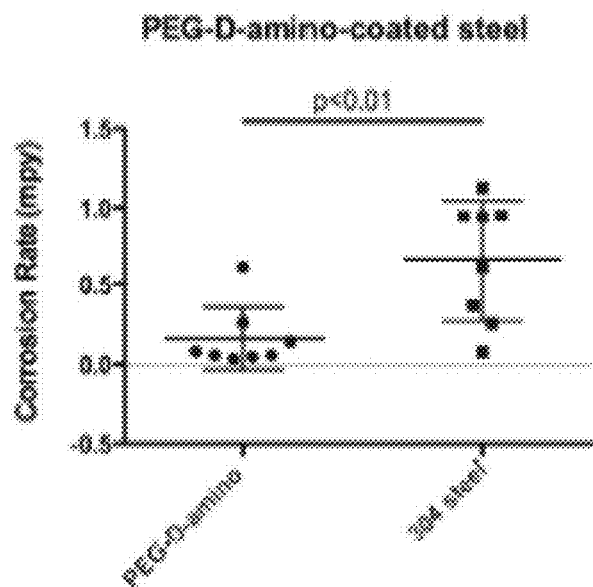


FIG. 31B

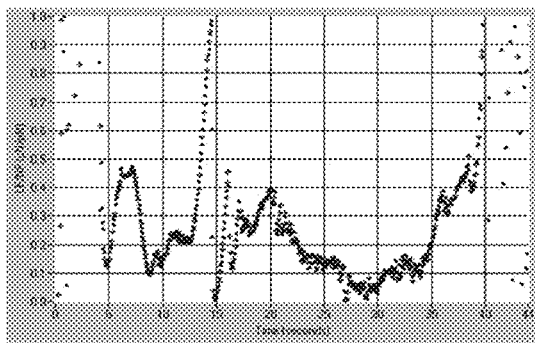


FIG. 32A

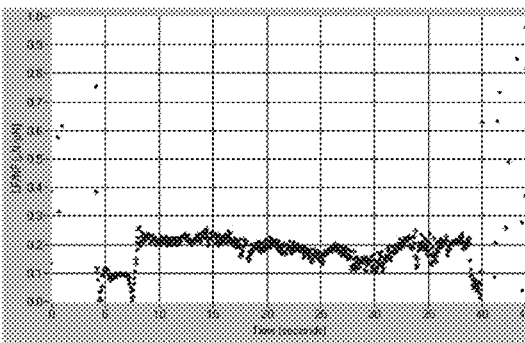


FIG. 32B

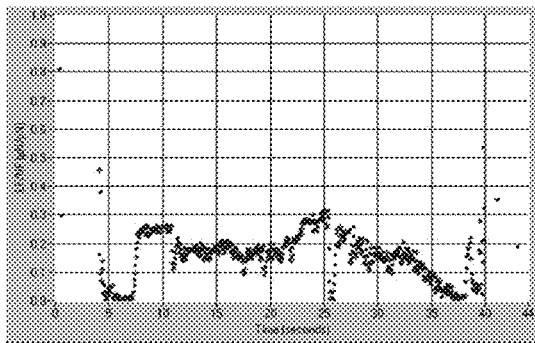


FIG. 32C

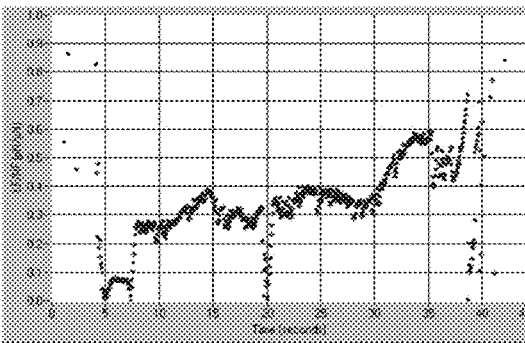


FIG. 32D

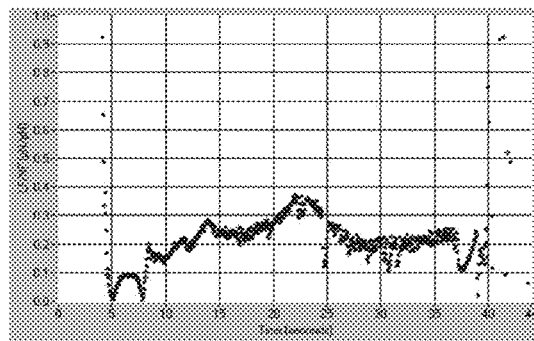


FIG. 32E

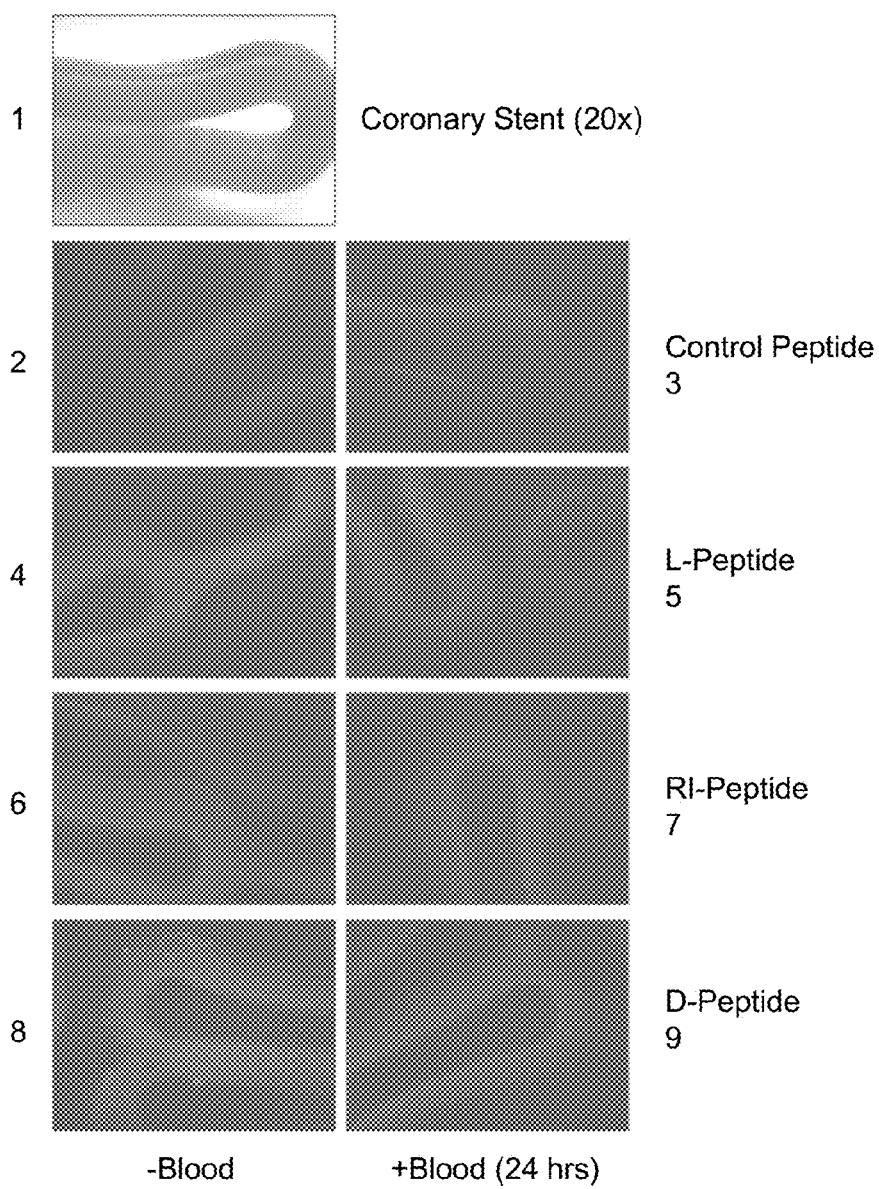


Fig. 33A

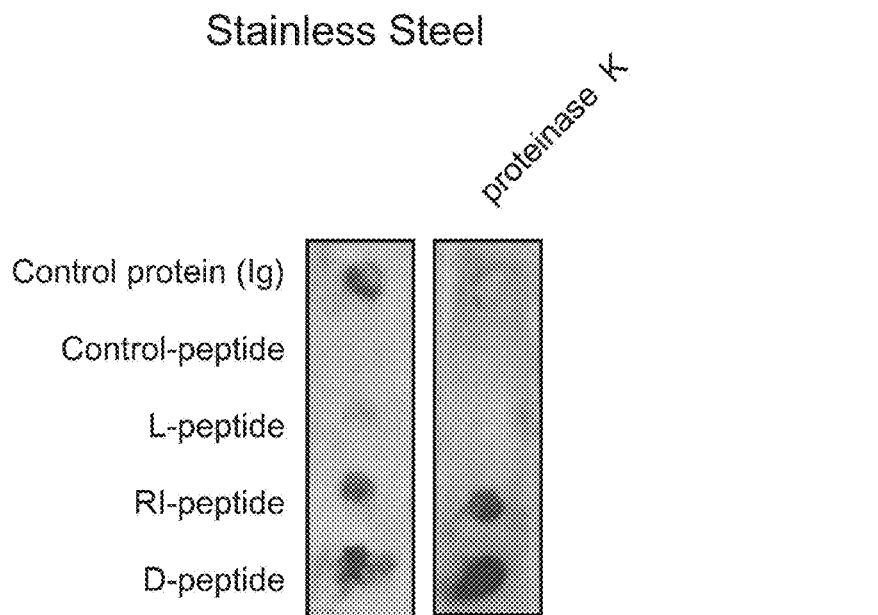


Fig. 33B

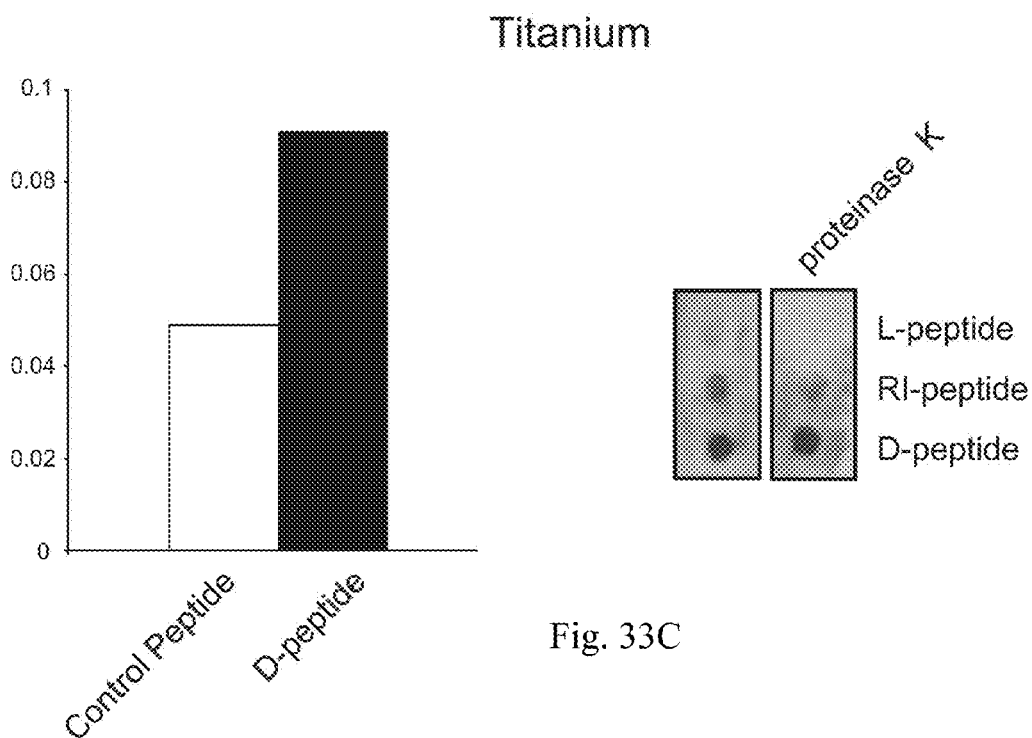


Fig. 33C

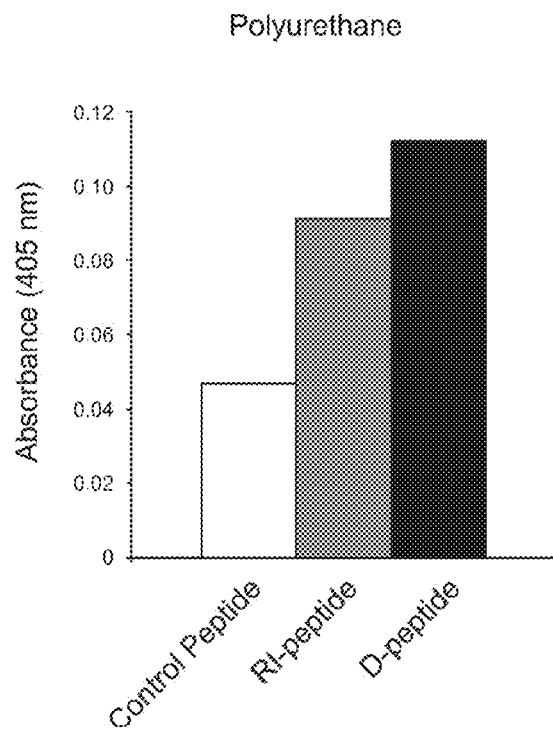
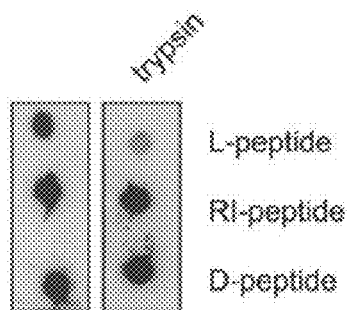


Fig. 33D

A. Polysulphone (dialysis membrane)



B.

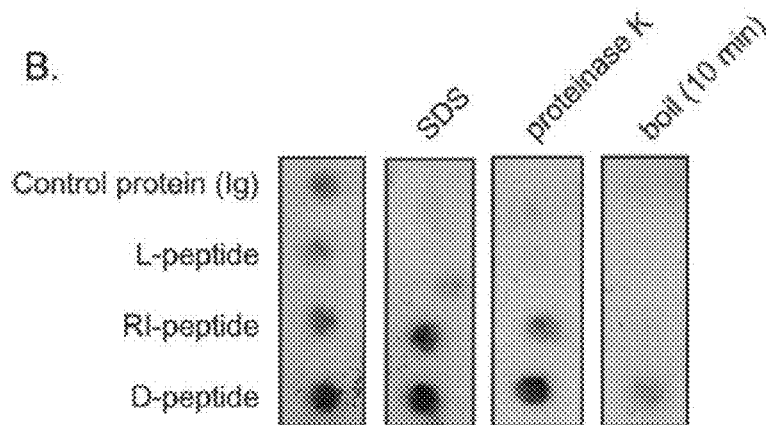


Fig. 33E

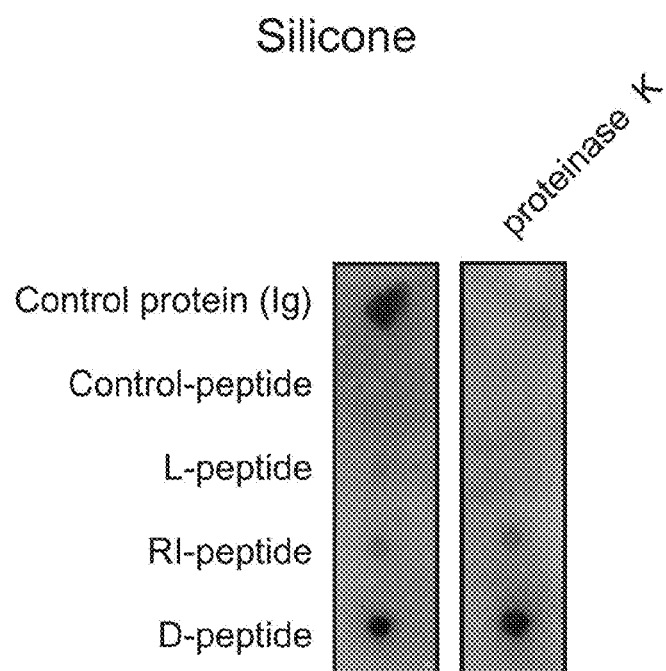


Fig. 33F

Stainless Steel

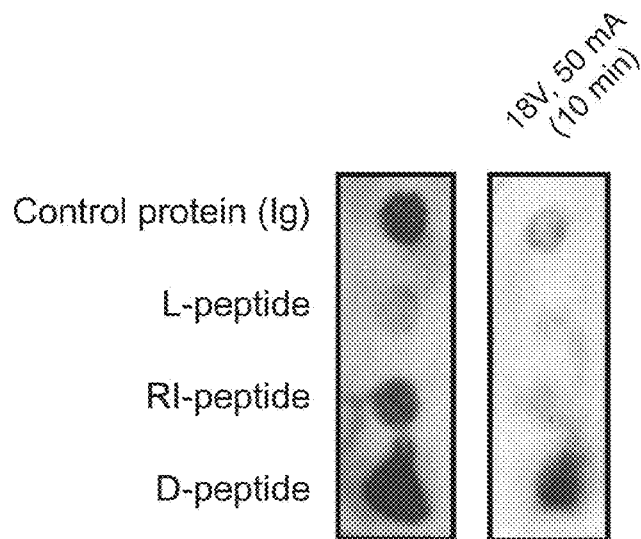


Fig. 34

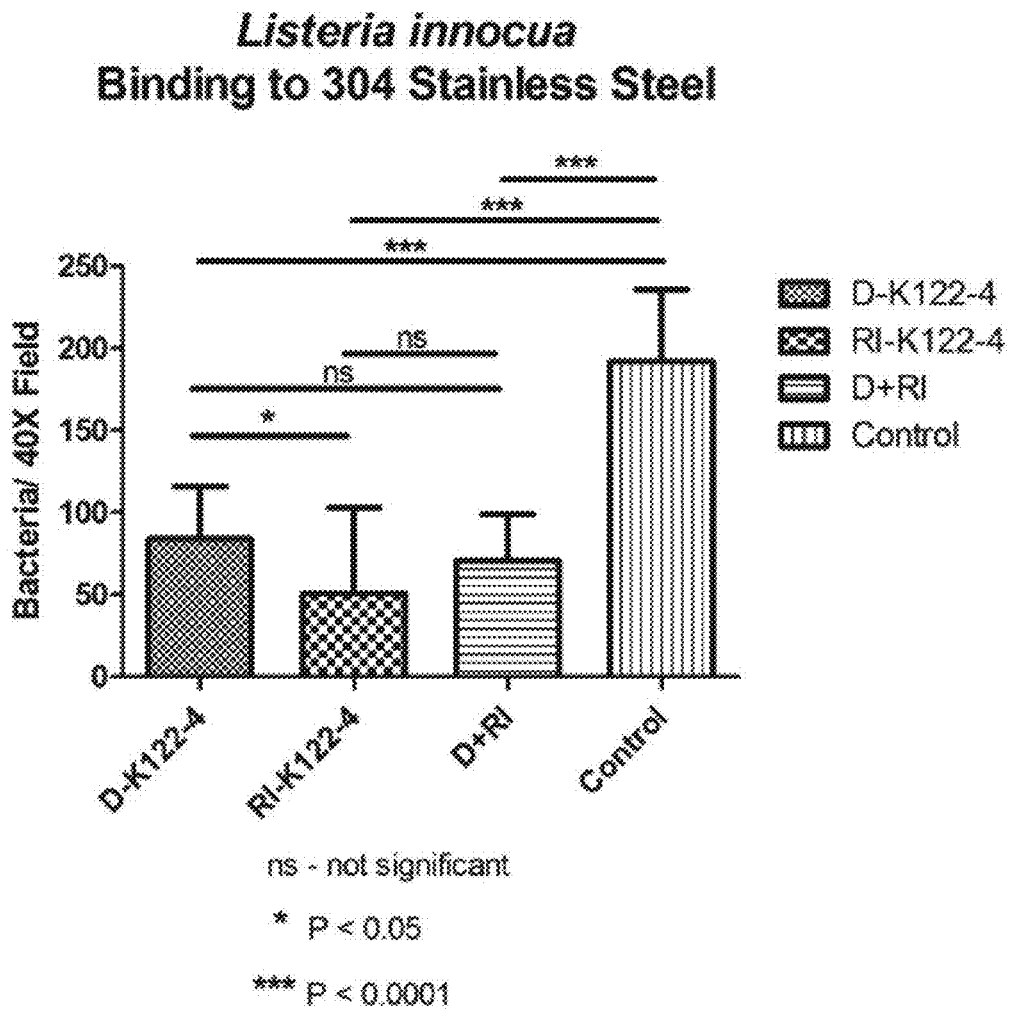
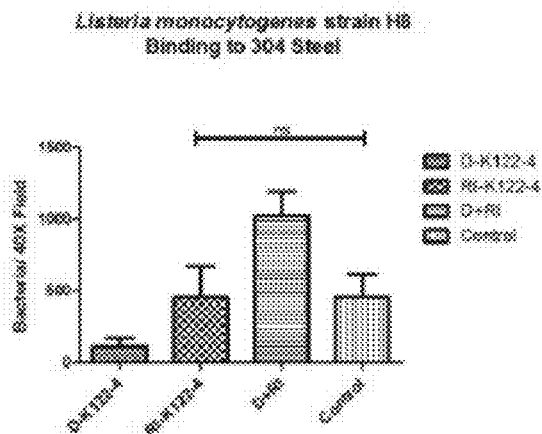
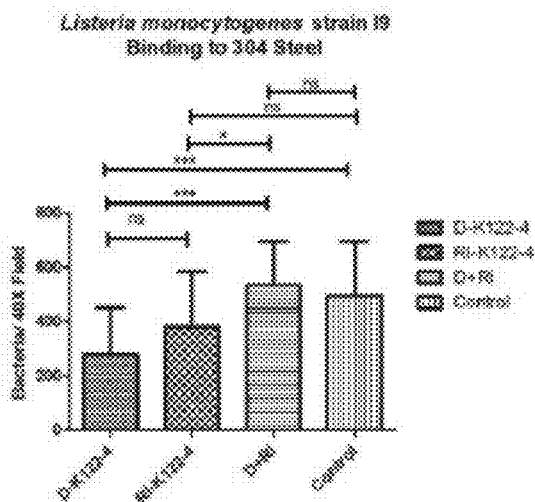


Fig. 35A



RI and Control are not significantly different (ns)
All other samples are highly significant $P < 0.001$

Fig. 35B



ns = not significant
* = $P < 0.05$
** = $P < 0.0001$

Fig. 35C

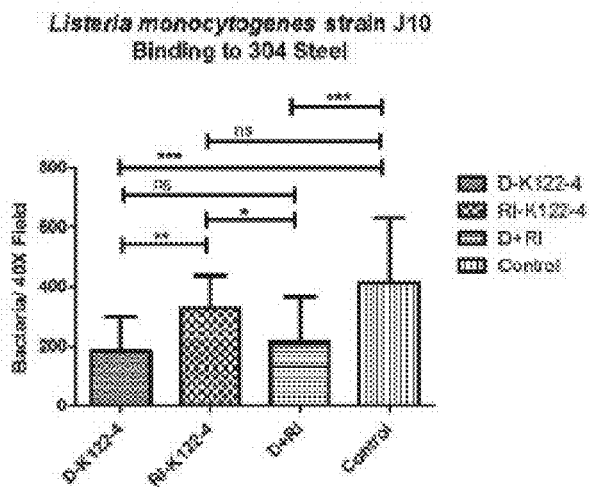


Fig. 35D

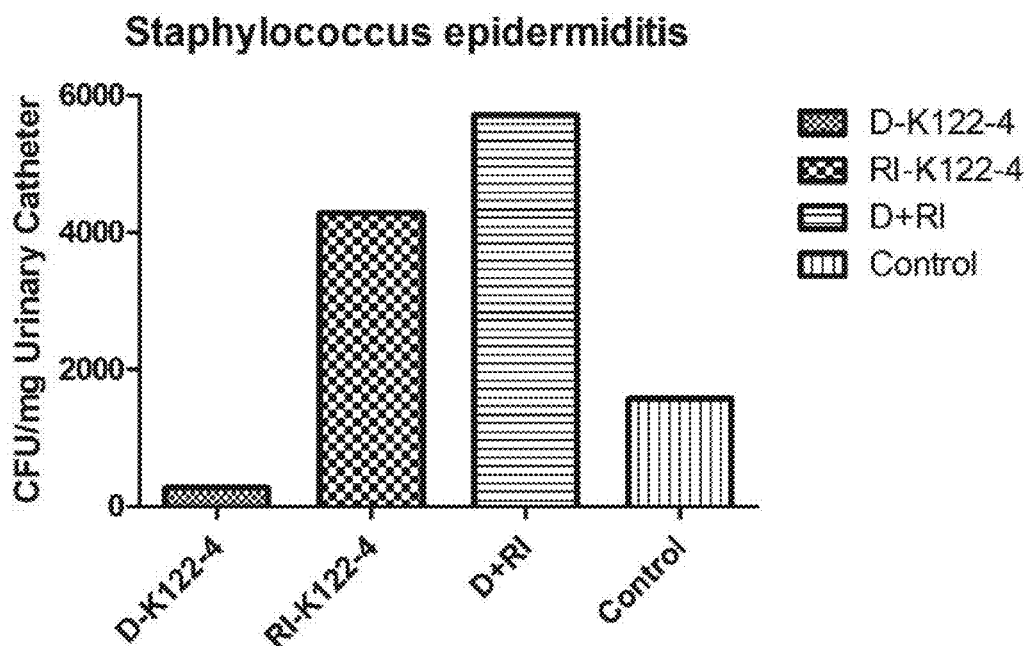


Fig. 36A

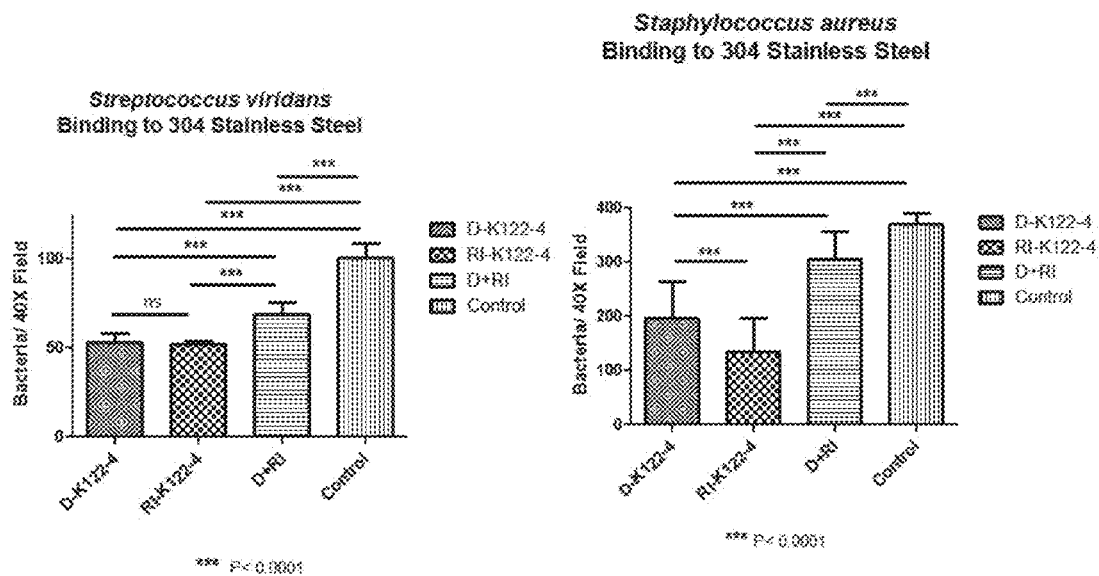


Fig. 36B

Fig. 36C

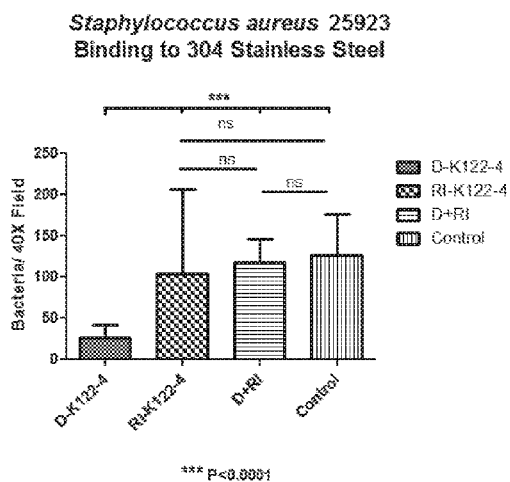


Fig. 36D

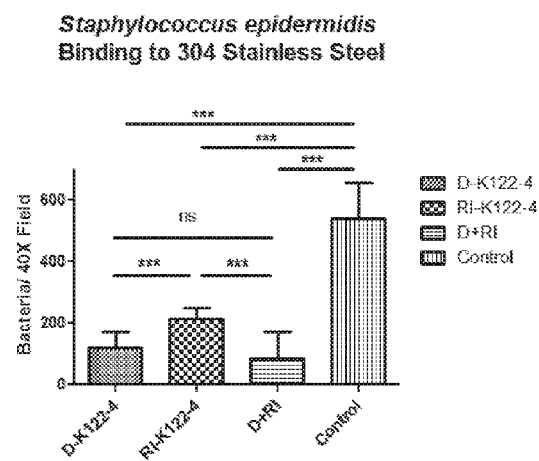


Fig. 36E

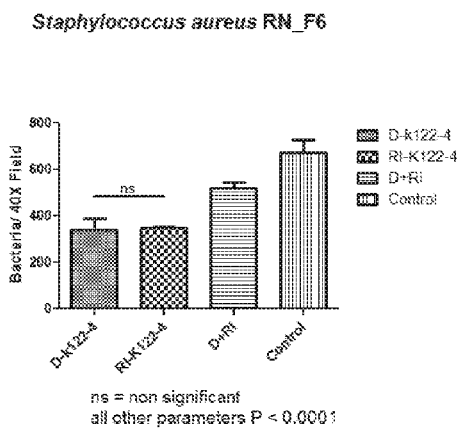


Fig. 36F

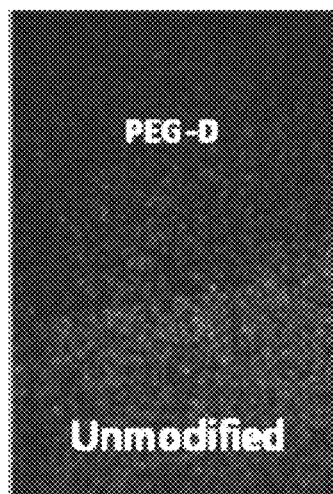


FIG. 37A

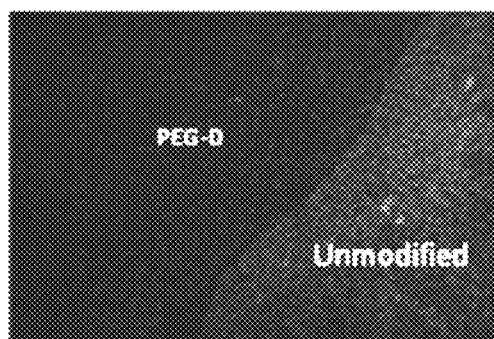


FIG. 37B

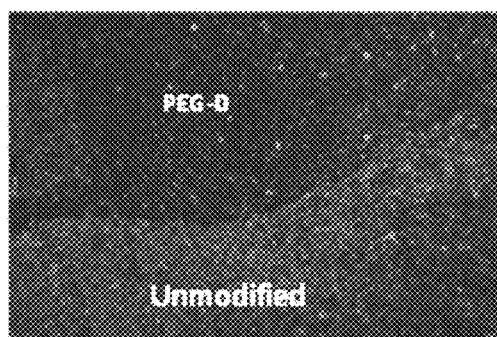


FIG. 37C

SURFACE COATED STRUCTURES AND METHODS

This application is a U.S. National Stage of International Patent Application No. PCT/CA12/00360, filed on Apr. 13, 2012, now U.S. Pat. No. 9,096,775 which claims the benefit of U.S. application Ser. No. 13/087,257, filed on Apr. 14, 2011, which claims the benefit of, continuation in part, U.S. application Ser. No. 12/899,958, filed on Oct. 7, 2010, now U.S. Pat. No. 8,961,984 which claims the benefit of U.S. Provisional Patent Application No. 61/249,934, filed on Oct. 8, 2009, which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to the field of material coatings that employ a D-form or retro-inverso Type IV *P. aeruginosa* (T4P) pilin peptide, optional conjugated to a polyethylene moiety, and to methods and applications thereof.

BACKGROUND

Bacterial Type IV pili are essential for host colonization and virulence for many Gram negative bacteria, and may also play a role in pathogenesis for some Gram positive bacteria. Type IV pili extend from the bacterial surface and mediate specific adherence to biotic and abiotic surfaces. The pili binding domain responsible for this binding is encoded within a 12-17 disulfide loop region located in the C-terminal region of the protein, and synthetic peptides containing this region only, e.g., a disulfide-loop peptide composed of residues 128-144 from the *Pseudomonas aeruginosa* Type IV pilin, have been shown to bind to biotic and abiotic surfaces.

The present inventors and colleagues have recently shown that pilin-derived protein nanotubes (PNTs) bind to stainless steel with high affinity, and the binding event was shown to be C-terminal tip-associated through competitive inhibition of PNT binding by synthetic peptides corresponding to the Type IV pilin peptide binding domains. (Yu, B. et al., J. Bionanoscience, 1:73-83 (2007)). It was then further demonstrated by the present inventors and colleagues that pilin peptides derived from the C-terminal receptor binding domain, when bound to abiotic surfaces such as stainless steel, tin, aluminum, titanium, chromium, plastic, glass, silicate, ceramics, and mixtures thereof, were able to inhibit bacterial biofilm formation on the coated surfaces (U.S. 20080287367).

More recently, the inventors discovered that binding of synthetic pilin peptide containing a disulfide loop derived from the C-terminal receptor binding protein of Type IV *P. aeruginosa* (T4P) pilin to some metals significantly enhances certain purely surfacial properties of the metal, that is, properties independent of biofilm formation, and in some metals, alters the electronic properties of the surfaces in ways that can be exploited, for example, in biosensor applications (U.S. Ser. No. 12/899,958).

SUMMARY OF THE INVENTION

In one aspect, the invention includes a method of treating a pipe to reduce the frictional drag of fluid flowing through the pipe, by the step of introducing into the pipe, a liquid carrying a conjugate of (i) a D-form or retro-inverso synthetic pilin peptide containing a disulfide loop derived from

the C-terminal receptor binding protein of Type IV *P. aeruginosa* (T4P) pilin and containing 0-10 additional residues on either or both the N- or C-terminal side of the loop and (ii) a polyethylene glycol moiety, in an amount of conjugate effective to reduce the frictional drag of fluid flowing through the pipe by attachment of the pilin peptide to the walls of the pipe.

The pilin peptide conjugate may be a D-form pilin peptide having a polyethylene glycol moiety covalently attached at one of both of the C- or N-termini of the peptide. The polyethylene glycol moiety in the peptide conjugate may have a molecular weight between 0.2 and 500 kDal. The pilin peptide may have the sequence identified as SEQ ID NO:10, including exemplary sequences identified as SEQ ID NOS: 3, 4, or 9.

The interior surface of the pipe to which the pilin peptide conjugate is attached may be a metal, polymer, ceramic or silicate surface. Exemplary metals include stainless steel, carbon steel, titanium, copper, brass, tin, iron, silver, and magnesium and alloys thereof.

The amount or surface density of pilin peptide conjugate attached to pipe's interior wall may be sufficient to reduce the rate of corrosion of a corrodible metal, such as stainless steel, and/or sufficient to reduce the frictional drag of a fluid, e.g., a sand-containing slurry introduced into the pipe.

The pilin peptide conjugate may be introduced into the pipe by periodically adding the peptide conjugate to fluid introduced into the pipe during normal operation.

In another aspect, the invention includes an article having an exposed metal, polymer, ceramic or silicate surface or surfaces coated with a conjugate of (i) a D-form or retro-inverso synthetic pilin peptide containing a disulfide loop derived from the C-terminal receptor binding protein of Type IV *P. aeruginosa* (T4P) pilin and containing 0-10 additional residues on either or both the N- or C-terminal side of the loop and (ii) a polyethylene glycol moiety, in an amount of conjugate sufficient to reduce the coefficient of friction of the exposed surface(s). Exemplary pilin peptides are as indicated above.

The exposed surface(s) of the article may be metal surfaces, such as surfaces of stainless steel, carbon steel, titanium, copper, brass, tin, iron, silver, and magnesium and alloys thereof, and the peptide conjugate coating the surfaces may be present in an amount sufficient to reduce the rate of corrosion of the surfaces, and/or to reduce the frictional drag of a fluid flowing through the pipe.

In one embodiment, the article is a microfluidics channel having a width dimension of 100 microns or less, whose interior wall surface is coated with the peptide conjugate and the peptide conjugate coating the surface is present in an amount sufficient to reduce the frictional drag of a fluid flowing through the channel.

In a second embodiment, the article includes sand particles whose outer surfaces are coated with a sufficient amount of the peptide conjugate to reduce the frictional drag of a slurry of the coated particles flowing through a pipe.

In a third embodiment, the article is a machine having moving parts whose peptide-coated surfaces are in moving contact with one another.

In a fourth embodiment, the article is an electronic device or component having exposed metal surfaces coated with the peptide conjugate.

In a fifth embodiment, the article is an orthopedic implant device having a stainless steel or titanium surface, and the peptide conjugate coating the surfaces is present in an amount sufficient to inhibit biofilm formation on the coated surfaces.

In yet another aspect, the invention includes an article composed of (i) an element having an exposed surface that can serve as a substrate for biofilm formation by a non-*Pseudomonas* bacteria under ordinary conditions of use, and (ii) bound to said surface, a D-form or a retro-inverso synthetic pilin peptide containing a disulfide loop derived from the C-terminal receptor binding protein of Type IV *P. aeruginosa* (T4P) pilin and containing 0-10 additional residues on either or both the N- or C-terminal side of the loop, and optionally, a polyethylene glycol moiety covalently attached to the peptide, in an amount effective to inhibit biofilm formation by such non-*Pseudomonas* bacteria. Exemplary bacteria inhibited include *Listeria* or *Staphylococcus* bacteria,

In still another aspect, the invention includes an improvement in a method of treating a metal material having surfaces with exposed grain boundary regions, to reduce the rate of corrosion or the material. The improvement involves contacting exposed surfaces boundary regions in the material with a D-form or retro-inverso synthetic pilin peptide containing a disulfide loop derived from the C-terminal receptor binding protein of Type IV *P. aeruginosa* (T4P) pilin and containing 0-10 additional residues on either or both the N- or C-terminal side of the loop, and optionally, a polyethylene glycol moiety covalently attached to the peptide, in an amount effective to inhibit the rate of corrosion of the material. Exemplary metals include stainless steel, carbon steel, titanium, copper, brass, tin, iron, silver, and magnesium and alloys thereof. Exemplary pilin peptide and pilin peptide conjugates are as above.

The amount of peptide or peptide conjugate bound to the metal may be effective to change the electron work function of exposed grain-boundary regions by at least 0.3 eV EFW units and to increase the hardness of exposed grain-boundary regions, as measured by nano-indentation produced striking the metal surface with the tip of an atomic force microscope with a given force, by at least 20%. Where the metal is stainless steel, the amount of bound peptide or peptide conjugate bound may be effective to reduce the rate of corrosion of the coated surface, as measured by the corrosion current across the surface, by at least about 30%.

Where the metal material is porous or reticulated, the contacting step may be carried out to bind the pilin peptide to internal surfaces defined by pores or reticulations in the material. Where the metal material has exposed grain-boundary regions, the contacting step may be effective to bind the pilin peptide selectively to said grain boundary regions, thus enhancing the hardness and corrosion resistance of the metal surface by preferentially protecting the surface at its grain boundary regions.

Also disclosed is a method of inhibiting an inflammatory response against a medical device that is designed to be implanted in a subject. The method includes, prior to implanting the device, coating exposed surfaces of the device with a synthetic pilin peptide containing (i) a disulfide loop derived from the C-terminal receptor binding protein of Type IV *P. aeruginosa* (T4P) pilin, (ii) 0-10 additional residues on either or both the N- or C-terminal side of the loop, and (iii) composed of D-amino acids in a retro-inverso (RI) form, and optionally having, a polyethylene glycol moiety covalently attached to the peptide. Preferred peptides and peptide conjugates are as indicated above.

In another aspect, the invention includes a biosensor device for detecting an analyte. The device comprises (a) a conductive metal substrate having a (i) biosensor surface (ii) bound to the substrate surface, a D-form (RI) synthetic pilin

peptide containing a disulfide loop derived from the C-terminal receptor binding protein of Type IV *P. aeruginosa* (T4P) pilin and containing 0-10 additional residues on either or both the N- or C-terminal side of the loop, and optionally having, a polyethylene glycol moiety covalently attached to the peptide, and (iii) a receptor covalently attached, directly or indirectly, to the pilin peptide, and (b) a detector for detecting a change in electrical property across the substrate surface in response to binding of an analyte-related ligand to the surface-bound receptor.

The biosensor substrate may be formed of stainless steel, and the pilin peptide bound thereto may be a D-form pilin peptide, optionally conjugated to a polyethylene glycol moiety. In one embodiment, a combination of pilin peptides may be employed, for example, using a PEG-D-pilin peptide conjugate to reduce non-specific interactions with the sample compounds, and an RI-pilin peptide (with or without a PEG moiety) to attach the target ligand to the biosensor surface, either directly by covalent attachment of the ligand to the peptide or indirectly, as described below.

The receptor may covalently attached to the pilin peptide through a coiled coil E/K coil pair, one of which is covalently attached to the pilin peptide, and the other of which is attached to the receptor.

In still another aspect the invention includes a method of covalently attaching a compound to one or more exposed surfaces of a substrate formed of stainless steel, tin, iron, or titanium, by the steps of (a) contacting exposed surface(s) of the substrate with a D-form synthetic pilin peptide containing a disulfide loop derived from the C-terminal receptor binding protein of Type IV *P. aeruginosa* (T4P) pilin and containing 0-10 additional residues on either or both the N- or C-terminal side of the loop, and optionally having a polyethylene glycol moiety covalently attached to the peptide, thereby to bind the pilin peptide covalently to the exposed surface(s), and (b) before or after said contacting and binding, covalently attaching the compound to the pilin peptide or, alternatively, to the PEG moiety in a PEG-pilin-peptide conjugate. Preferred pilin peptides and peptide conjugates are as described above.

Where the substrate surfaces have exposed grain boundary regions, the contacting step is effective to preferentially localize the compound at exposed grain boundary regions. Where the material is porous or reticulated, the contacting step may be effective to bind the pilin peptide to internal surfaces defined by pores or reticulations in the material.

Also disclosed is a substrate whose surface has bound thereto, (i) a D-form synthetic pilin peptide containing a disulfide loop derived from the C-terminal receptor binding protein of Type IV *P. aeruginosa* (T4P) pilin and containing 0-10 additional residues on either or both the N- or C-terminal side of the loop, and optionally having a polyethylene glycol moiety covalently attached to the peptide, and (ii) a compound that is covalently attached, directly or indirectly, to the pilin peptide or to the PEG moiety in a PEG-pilin-peptide conjugate.

The substrate may be a metal, such as stainless steel, carbon steel, titanium, copper, brass, tin, iron, silver, and magnesium and alloys thereof, and may function as a biosensor electrical response element, where the compound is an analyte receptor molecule directly attached to the pilin peptide through a covalent linkage, or indirectly attached to the pilin peptide through a K/E coiled/coil pair, where one of the coils is covalently attached to the pilin peptide, and the other coil is covalently attached to the compound. A stainless steel substrate may have an altered electron work function by virtue of the pilin peptide bound to its surface.

These and other objects and features of the invention will become more fully apparent when the following detailed description of the invention is read in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1 A-1 D show the sequences of C-terminal receptor binding domains of Type IV *P. aeruginosa* (T4P) pilin in a number of bacterial genus/species/strains;

FIGS. 2A and 2B are plots showing adhesive force measurements made on uncoated stainless steel or stainless steel coated with L-form K122-4 (2A) and PAO (2B) pilin peptide;

FIGS. 3A and 3B are plots showing electron work functions (EWF) for uncoated stainless steel and stainless steel coated with L-form K122-4 (3A) and PAK (3B) pilin peptide;

FIG. 4 shows EWF measurements with coated and uncoated stainless steel taken over a two-month period;

FIG. 5 shows a force-displacement curve of peptide-coated and uncoated stainless steel under a 800 nM load;

FIGS. 6A and 6B show the results of nano-indentation tests conducted at 20 μ N (6A) and 50 μ N (6B) loads;

FIGS. 7A and 7B are plots of displacement measurements at increasing loads for peptide coated (PAO in 7A and K122-4 in 7B) and uncoated stainless steel;

FIGS. 8A and 8B are conductance AFM surface maps of current flow between an uncoated (8A) and T4P17 pilin peptide coated (8B) stainless steel substrate and an AFM tip;

FIGS. 9A-9B are measurements of corrosion characteristics in coated and uncoated stainless surfaces, showing that pilin-coated stainless steel has a corrosion current (I_{corr}) that is significantly below that for uncoated stainless steel (9A), that pilin-coated and uncoated stainless steel do not have significantly different corrosion potentials (E_{corr}) (9B);

FIGS. 10A and 10B are plots showing that pilin-coated stainless steel has a significantly lower corrosion rate than uncoated stainless steel (10A) and a significantly higher resistance to polarization compared to uncoated stainless steel (10B);

FIG. 11 shows that the corrosion inhibitory effect of pilin peptide binding to a metal surface can be reversed when the pilin peptide is conjugated to another peptide, in this case, a leucine-zipper type E coil or an E/K coiled coil;

FIGS. 12A-12C are photographs showing the visual effects of corrosion on stainless steel samples that are either uncoated (12A), coated with a pilin peptide (12B), or coated with a pilin peptide having an coil-coil duplex attached to the pilin;

FIG. 13 shows that bound pilin peptide is not displaced from stainless steel surfaces in a competitive binding assay employing increasing amounts of exogenous peptide;

FIG. 14 is a XPS plot of an uncoated and K-122-4 pilin peptide coated stainless steel sample;

FIGS. 15A and 15B show adhesive force measurements on a stainless steel sample coated with both L- and D-forms or pilin peptides (15A) and the EWF force measurements on a stainless steel sample also coated with the same L- and D-forms of pilin peptides (15B);

FIGS. 16A and 16B demonstrate the ability of pilin peptide (D-form) to bind tightly to a stainless steel stent (16A) and to a glass surface (16B);

FIGS. 17A and 17B are bar graphs showing the relative resistance to protease digestion in L-amino acid and D-amino acid pilin bonded to a stainless steel sample;

FIGS. 18A and 18B are schematic illustrations showing the operation of a biosensor device constructed according with one embodiment of the invention;

FIG. 19 illustrates analyte-binding steps in a biosensor in which the analyte-binding agent R is attached directly to the biosensor surface through a pilin peptide;

FIG. 20 illustrates analyte-binding steps in a biosensor in which the analyte-binding agent R is attached to the biosensor surface through a pilin peptide coiled-coil complex;

FIG. 21 shows different detection configurations in the biosensor of the invention;

FIGS. 22A and 22B are cyclic voltammetry plots recorded in a biosensor device before and after binding analyte binding to the receptor, where 22B is an enlargement of the rectangular panel in FIG. 22A;

FIGS. 23A and 23 B illustrate an analyte detection device constructed according to a more general embodiment of the invention;

FIGS. 24A-24D are bar graphs showing binding of T4P17 pilin peptide to stainless steel (24A), a coronary stent (24B), a Foley (latex) catheter (24C), and a central venous (silicone) (24D) catheter;

FIGS. 25A and 25B are a Western blot (25A) and bar graph plot (25B) showing the effect of D-pilin peptide coating on a PBMC immunological response to an uncoated and pilin-coated titanium or stainless steel surface;

FIGS. 26A and 26 B show a Western blot (26A) and bar graph plot (26B) showing the effect of D-pilin peptide coating on a human macrophage immunological response to an uncoated and pilin-coated titanium or stainless steel surface;

FIGS. 27A-27D show characteristics of stainless steel coated with various forms of a K122-4 pilin peptide the borg sEWF of K-122-4, for EWF (27A and 27B), nanoindentation (27C), and corrosion rate (27D);

FIGS. 28A-28C are plots showing electron work function (EWF) of uncoated titanium or titanium coated with D-form, retro-inverso, or D-form-PEG pilin peptides;

FIG. 29 shows surface microindentation effects on a titanium surface either uncoated or coated with D-form, retro-inverso or D-form-PEG pilin peptides;

FIGS. 30A-30E show bulk microindentation effects on a titanium plate at the force levels indicated;

FIGS. 31A and 31B show adhesive force measured on a titanium plate (FIG. 31A), and corrosion rate (31B) on a stainless steel plate, both for uncoated or coated with PEG-D-form pilin peptide;

FIGS. 32A-32E are representative coefficient-of-friction profiles of uncoated titanium (31A), titanium coated with PEG-D-form pilin peptide (32B), titanium coated with retro-inverso form of pilin peptide (32C), titanium coated with D-form pilin peptide (32D), and titanium coated with a combination of retro-inverso and D-form pilin peptide (32E);

FIGS. 33A-33F present data comparing the binding of L-pilin peptide, RI-pilin peptide, and D-form peptide to various materials, including stainless steel (33A, Parts 1-9 and 28B), titanium (33C, parts A and B), polyurethane (33D), polysulphone (33E, parts A and B), and silicone (33F);

FIG. 34 shows relative loss of bound control (Ig) peptide, L-form pilin peptide (L), RI pilin peptide (RI), and D-form pilin peptide from a stainless steel surface with application of a 50 mA current for 10 minutes;

FIGS. 35A-35D are plots showing the extent of biofilm inhibition of various strains of *Listeria innocua* (35A) and three strains of *Listeria monocytogenes* (35B-35D) bacteria

on stainless steel plates coated with D-form pilin peptide, RI pilin peptide, or D+RI pilin peptides, or uncoated control;

FIGS. 36A-36F are plots showing the extent of biofilm inhibition of various species of *Staphylococcus* bacteria on stainless steel plates coated with D-form pilin peptide, RI pilin peptide, or D+RI pilin peptides, or uncoated control; and

FIGS. 37A-37C show the bacterial inhibition effects of PEG-D-pilin peptide applied to stainless steel as a very small drop.

DETAILED DESCRIPTION

I. Definitions

A “pilus” is a hairlike appendage found on the surface of many bacteria.

A pilin is the general term for the protein subunit of a pilus.

“Type IV *P. aeruginosa* (T4P) pilin” or “T4P pilin peptide” or “pilin peptide” refers to the pilus structures that *P. aeruginosa* bacteria use to generate motile forces, by adhering the distal end of the pilus to a biotic or abiotic surface, and contracting the pilus to pull the bacteria forward. All Type IV *P. aeruginosa* (T4P) pili contain a C-terminal receptor binding region that, in oxidized form, contain a disulfide loop of that can be classified into either a 12-residue loop or a 7-residue loop. FIG. 1 shows the disulfide loop region in a number of bacterial species whose C-terminal pilin regions have been sequenced.

A “disulfide loop derived from the C-terminal receptor binding protein of Type IV *P. aeruginosa* (T4P) pilin” refers to a disulfide loop whose amino acid sequence corresponds to a known bacterial disulfide loop amino acid sequence, such as the *Pseudomonas aeruginosa*, strain PAK sequence identified by SEQ ID NO:4, or one of the sequences is formed as a permutation one or more amino acid variations in the disulfide loop sequences between two or more different bacterial species and/or strains, such as one of the sequences contained in SEQ ID NO: 10 which is formed as a composite of the disulfide loop sequences of the four *P. aeruginosa* strains PAK, PAO, PA82935, and K-122-4.

A “synthetic peptide” refers to a peptide that is formed by solid-phase or recombinant peptide synthesis.

“A substrate formed of stainless steel, tin, iron, or titanium” means a metal substrate formed of stainless steel, tin, iron, or titanium or mixtures of two or more of these metals, or a metal or non-metal substrate coated with stainless steel, tin, iron, or titanium or a mixture thereof. The substrate may contain minor proportions of other metals, particularly transition metals from rows 4-6 and columns 9-12 of the period table, including cobalt, nickel, copper, zinc, ruthenium, rhodium, palladium, silver, cadmium, osmium, platinum, gold, chromium (present in stainless steel), and mercury.

“Covalent attachment of a T4P pilin peptide to a metal substrate formed of stainless steel, tin, iron, or titanium” means that the pilin peptide is attached to the metal surface through a bond that (i) resists displacement by free pilin peptides, and (ii) has an altered electron work function (EWF) indicating a change in the surface e^0 orbitals of the material. Covalent attachment of a pilin peptide to such a metal substrate may also be characterized by a (i) change in surface adhesive force, (ii) change in surface hardness, (iii) change in conductance, and/or (iv) change in binding-energy peaks seen in X-ray photoelectron spectroscopy (XPS).

“Metal substrate with covalently bound compound” means a stainless steel, tin, iron, or titanium” substrate

having a T4P pilin peptide covalently bound to the substrate surface, and a compound other than another portion of a pilin protein covalently bound, either directly or indirectly, to the pilin peptide. A compound is indirectly covalently bound to the T4P pilin peptide when it is linked to the pilin peptide through a high-affinity binding pair, such as a coiled-coil leucine-zipper pair, a biotin-avidin pair, or the like, where one of the members of the pair is covalently linked to the pilin peptide and the other, covalently linked to the compound.

“Exposed grain-boundary regions” of a metal substrate refer to surface regions of the substrate at which grain boundaries occur, that is, at the interface of two polycrystalline orientations of the metal atoms forming the substrate.

“Preferential localization of a pilin peptide at exposed grain boundary regions” means that the pilin peptide, and any compound covalently attached to the pilin peptide, has a greater concentration of molecules and/or thickness of surface coating at grain boundary regions than at exposed surface regions between grain boundaries.

“L-form T4P pilin peptide” or “L-form pilin peptide” refers to a T4P pilin peptide composed of L-enantiomeric amino acid residues (L-amino acid residues).

“D-form T4P pilin peptide” or “D-form pilin peptide” refers to a T4P pilin peptide composed primarily or exclusively of D-enantiomeric amino acid residues (D-amino acid residues). Specifically, a D-form T4P pilin peptide contains greater than 50% D-amino acid residues, and preferably all D-amino acids, with any remainder residues being L-amino acids. D-form T4P pilin peptides are protease resistant, and any L-enantiomeric amino acids in the peptide should be positioned within the pilin peptide sequence such that they do not significantly compromise this protease resistance. These positions can be determined by forming a series of D-form pilin peptides having a single L-amino acid substitution at each possible position, and testing the single-substitution peptides for resistance against a variety of proteases.

Retro-inverso T4P pilin peptide” or “retro-inverso pilin peptide” or “RI pilin peptides” refers to a T4P pilin peptide having an inverted sequence of D-enantiomeric amino acid residues, which will position the amino acid side chains in the correct relative positions, as described below. Retro-inverso T4P pilin peptides are protease resistant.

“Polyethylene glycol” or “PEG” refers to linear or branched polymers of ethylene glycol, and may include as few as 5 monomeric units and up to many thousand units, with a range of molecular weights between 0.2 kDaltons to 500 kDaltons or more, where the specified size refers to average molecular weight of the PEG. “Polyethylene glycol moiety” refers to the polyethylene glycol portion or moiety of a “conjugate” of a pilin peptide, as defined above, and polyethylene glycol. The conjugate is typically a covalent conjugate in which a polyethylene glycol moiety is covalently attached to the C-terminal and/or N-terminal amino acid of the pilin peptide.

II. Pilin Peptides

FIGS. 1A-1D show the sequences for the C-terminal pilin peptide regions containing a disulfide loop for a variety of bacterial genus/species/strains for which sequence information is available. The sequences given include the disulfide loop sequence (beginning and ending with a cysteine residue (C) and including in some cases up to five or more residues on either side of the loop. The single-letter amino acid designations are according to standard convention. In the

normal oxidized form of the peptides, the peptides contain a disulfide bridge between cysteine residues.

The synthetic peptide employed in the present invention includes or is derived from one or more of the sequences shown in FIGS. 1 A-1 D. Where the sequence includes one of the sequences shown, it may include that disulfide loop alone, or the loop may additionally include up to ten, preferably five or fewer residues at either or both of the N-terminal or C-terminal side of the loop, where the additional non-loop residues typically include or are derived from one or more of the adjacent non-loop sequences. More generally, the sequences of both loop and non-loop regions may be derived from two or more sequences by aligning the sequences, preferably sequences having the same or nearly the same number of residues in the disulfide loop. For example, in FIG. 1A, four of the peptides, corresponding to *P. aeruginosa* strains PAO (SEQ ID NO: 3), PAK (SEQ ID NO:4), PA82935 (SEQ ID NO:7), and K122-4 (SEQ ID NO:9) contain 14-mer disulfide loops. By aligning the disulfide loop sequences from the four *P. aeruginosa* strains in FIG. 1A, a combined sequence K/A S/T-C-T/K/A-S/T-D/T/N-QN/A-D/E-E/IP/A/N-Q/M/K-FA'-I/T/R/L-P-K/N-G/T--C-S/D/T/Q/N-K/N/D/T (SEQ ID NO: 10) emerges. This 17-residue peptide (also referred to generically as T4P17) includes 14 disulfide loop residues, a single upstream (N-terminal side) non-loop residue and two downstream non-loop residues. Exemplary sequences within this sequence include the actual four different sequences from which SEQ ID: 10 is derived, i.e., the sequences corresponding to the PAK (SEQ ID NO:3), PAO (SEQ ID NO: 4), PA82935, (SEQ ID NO:7), and K-122-4 (SEQ ID NO: 9).

As another example, the two *P. aeruginosa* strains G7-09 (SEQ ID NO:1) and PA110594 (SEQ ID NO:2), form a composite sequence S/T-I-D-W-G/A-C-A/T-S-D/A-S-N-A-V/T-S/--S--G/A-T-D/A-R/Q-N/G-M/F-P/T-A/G/L-M-T/A-A-G-T/S-L/V-P-A/Q-R/E-F-A-P-S/A-E/Q-C-R (SEQ ID NO:21)

Once a pilin peptide sequence is selected, it can be synthesized by standard recombinant or solid-phase synthesis. E-coil PAK(128-144)ox, for example, was expressed recombinantly from a pRLD-E plasmid, where the PAK (128-144)ox DNA sequence was spliced in-frame with the E-coil utilizing synthetic oligonucleotides and expressed in *E. Coli* strain BL-21 according to known techniques (see, for example, Giltner et al., Mol. Microbiology (2006) 59(4): 1083 and references cited therein). The expressed peptide was purified by metal affinity chromatography, the purity and formation of the disulfide bridge was confirmed by mass-spectroscopy and N-terminal protein sequencing.

In one general embodiment of the present invention, the pilin peptides are composed of D-amino acids, and as noted above, may contain one or more L-amino acids, as long as the L-amino acids are a minor fraction (fewer than 50% of the residues) and do not significantly compromise the protease resistance of the peptide. One purpose of including D-amino acids in the pilin peptide is to increase the peptide's resistance to proteolysis by one or more protease enzymes to which the peptide may be exposed. For example, *Pseudomonas* bacteria have a collection of proteases, including elastase, metallo proteases, and classic trypsin-like serine proteases, that require or target lysine and/or arginine residues in peptide cleavage. Thus, the pilin peptide could be synthesized to contain D-lysines, for example, at K136 and K140 in the K122-4 pilin peptide. Preferably, in making the peptide resistant to as many proteases as possible, the pilin peptide should be formed entirely from D-amino acids. Pilin peptides composed of all D-amino acids or a mixture of D-

and L-amino acids can be formed by conventional solid-phase methods utilizing activated D- or L-form amino acid reagents in the synthesis. (See, for example, Guichard, G., et al., Proc. Nat. Acad. Sci. USA Vol. 91, pp. 9765-9769, October 1994).

As will be seen below, D-form pilin peptides have important advantages over L-form pilin peptides as surface-coating agents. First, although the L-form T4P pilin peptide is effective in inhibiting biofilm formation caused by *Pseudomonas* bacteria, the D-form pilin peptide provides superior inhibition against biofilm formation by *Pseudomonas* bacteria, and dramatically stronger inhibition against biofilm formation by non-*Pseudomonas* bacteria, such as *Listeria* and *Staphylococcus* bacteria. Secondly, although the L-form T4P pilin peptide is effective to bind with high affinity to a variety of materials, including many metals, polymers, silicates, and ceramics, the D-form peptide in general provides a substantially more stable binding when the material is exposed to proteases and, in the case of conductive materials, when an electrical current is applied to the material.

In another general embodiment, the pilin peptides are composed of D-amino acids synthesized in a reverse-sequence direction, that is, in a carboxy to amine end direction, to produce a so-called retro-inverso (RI) pilin peptide. Retro-inverso (RI) form pilin peptides also have the advantage of greater resistance to proteases, and are thus advantageous in the applications described herein where the pilin-coated material is exposed to proteases, e.g., in a biological setting, or in an environment subject to bacterial growth. Methods for synthesizing RI-form peptides are detailed, for example, in Fletcher, M. D. and Campbell, M. M., Partially Modified Retro-Inverso Peptides: Development, Synthesis, and Conformational Behavior, Chem Rev, 1998, 98:763-795, which is incorporated herein by reference.

As will be seen below, the retro-inverso pilin peptides also have important advantages over L-form pilin peptides as surface-coating agents. First, they provides superior inhibition against biofilm formation by *Pseudomonas* bacteria, and dramatically stronger inhibition against biofilm formation by non-*Pseudomonas* bacteria, such as *Listeria* and *Staphylococcus* bacteria, although in general, the D-form pilin peptide produces a stronger inhibition effect on biofilm formation than does the RI peptide. Secondly, although the L-form T4P pilin peptide is effective to bind with a high-affinity to stainless steel, through a covalent-like bond characterized by an increased electron work function (EWF), greater hardness and corrosion resistance, the retro-inverso pilin peptide has been found to produce a significantly higher EWF of steel beyond that produced by the L-form peptide, and also significantly increased the material hardness, indicating also a greater resistance to corrosion over that seen with the L-form peptide.

In still another embodiment, the pilin peptide, and preferably D-form or retro-inverso form of the pilin peptide, is conjugated to a polyethylene glycol polymer linear or branched chain, typically by covalently linking an end of a polyethylene glycol linear or branched polymer to the N-terminal amine and/or C-terminal acid group of the peptide, using conventional peptide coupling methods, e.g., for forming amide, ester, ether or disulfide linkages between the peptide and PEG moiety, or a linkage containing an activation group. PEGs or PEGs with suitable reaction groups for use in conjugating to proteins are commercially available, e.g., from PEGTech, Analytical Ventura, and Thermo Scientific (branched PEGs) and the activating group may be, for

example, maleimide (typically, for reaction with peptide sulhydryl groups, vinyl sulfones, propionaldehyde, pyridyl disulfones, NHS ester, or iodoacetamide; PEGs with terminal amine, acid, hydroxyl, or sulhydryl reactive groups may be coupled to the peptide amine, acid, hydroxyl, or sulfur groups through known coupling reactions. The PEGs typically have an inert group, e.g., methoxy, at one end and an activated or reactive group at the other end. The polymer reagents have typical average molecular weights in a selected size range between 5 kDa to 40 kDa, corresponding to 100 to 1,000 repeat units in the polymer, although smaller or larger PEG are suitable. Pegylation of the proteins by the activated PEG reagents is carried out according to standard PEG coupling methods.

Advantages of a PEG-D-pilin peptide or PEG-RI-pilin peptide will be seen below. In general the pegylated form the D-form or RI pilin peptide provides the advantages noted above for the non-pegylated form of the D- or RI-form of the peptide, but with the additional advantages of significant reduction in frictional coefficient and greater inhibition of biofilm formation on a coated surface.

III. Treating Metal Surfaces

The invention includes, in one aspect, an improved method for treating stainless steel, tin, iron, or titanium metal material having surfaces with exposed grain boundary regions, to reduce the rate of corrosion of the material. The method may be used separately or in combination with one of a number of other anti-corrosion methods, such as passivation. The metal material may have a single exposed surface with grain-boundary regions, or a number of external surfaces to be treated, or contain pores or internal reticulations which are accessible from the external surface(s) of the material. As will be appreciated, the method is suitable to treating any stainless steel, tin, iron, or titanium metal material that is subject to chemical corrosion, e.g., in an oxidative atmosphere or by contact with corrosive liquids, such as basic or acidic liquids.

In practicing the method, the metal material may be first washed one or more times, e.g., in an ethanol bath, to remove contaminants. The material is then contacted with a solution of the pilin under conditions effective to covalently bind the pilin to the exposed surfaces of the material. In a typical treatment method, the material is placed in a solution of pilin peptide or PEG-pilin-peptide conjugate at a peptide or conjugate concentration between 2 $\mu\text{g/mL}$ and 50 $\mu\text{g/mL}$ pilin, e.g., 10 $\mu\text{g/mL}$, in an aqueous buffer, e.g., phosphate buffered saline, at a near-neutral pH, e.g., pH 7, and contacted with the solution for a period, e.g., 5-120 minutes, until a suitable coating of pilin peptides has formed.

Alternatively, the material to be coated may be sprayed with a pilin solution, and contacted with the sprayed solution in a high-humidity environment over a desired contact time, e.g., 5-120 minutes.

In still another embodiment, a pilin coating is applied to selected areas of the metal surface, e.g., in a microfabrication operation, or to selectively apply the peptide to exposed grain-boundary regions on the material. In this embodiment, a solution of the peptide is delivered to the exposed surface(s) of the material in an area-specific manner, e.g., by an ink-jet printer or the like.

IIIA. Treatment Method and Changes in Metal Surface Properties

This section describes exemplary methods for treating a metal surface to enhance its corrosion resistance, and studies conducted in support of the invention that demonstrate, in

addition to increased corrosion resistance, (i) reduced adhesive force of the treated surface, (ii) altered electron work function of the treated surface, (iii) increased hardness of the treated surface, (iv) reduced conductance, and (v) coating stability over a period of at least two months. The studies reported in this Section and in Sections IIIB and IIIC were carried out with the L-form pilin peptide, unless indicated otherwise. The studies and results reported in Section IIID were carried out with the D-form and retro-inverso pilin peptides and the PEG-conjugate of the D- and RI pilin peptides.

Sample Preparation

Commercial grade 304 2B finish plates (20 gauge) stainless steel sheets 1 mm thick were cut into samples with dimensions of 1 cm \times 1 cm. Samples were annealed at 1140 $^{\circ}$ C. for 1 hour in air and cooled in air. The surface was polished using sand papers of 120, 240, 320, 400, 600, and 800# grit, followed by a final polish with 1200# grit paper.

Aluminum and stainless steel samples with dimensions of 1 cm \times 1 cm \times 1 cm were polished using the polishing protocol described previously. Neither of these samples was annealed prior to polishing.

Coating Samples with Peptide or Monomeric Pilin

Stainless steel and aluminum samples were washed using a commercial dishwashing soap for 1 minute followed by rinsing with distilled water. Samples were then immersed in 95% ethanol with gentle agitation for 15 minutes, rinsed with distilled water, and immersed in reagent grade acetone for 1 minute. Samples were rinsed 5 times with distilled water and allowed to air dry. Samples were immersed in a phosphate buffered saline (PBS) solution containing 10 $\mu\text{g/mL}$ of peptide or peptide conjugate or monomeric pilin and were incubated at room temperature (RT) for 1 hour with gentle agitation. The solution was removed and samples were washed 6 times with distilled water and allowed to air dry.

Carbon steel samples were cleaned using the protocol described above, but were instead rinsed with 100% methanol following the acetone washing step and immediately immersed in 100% methanol to prevent rapid air corrosion that results when exposed to water. Peptide or peptide conjugate was dissolved in 100% methanol and a final concentration of 10 $\mu\text{g/mL}$ was used to immerse the carbon steel sample. Samples were incubated at RT for 1 hour with gentle agitation. Samples were washed 6 \times with 100% methanol and allowed to air dry.

Adhesive Force Measurements

The adhesive force between a standard Au-coated AFM silicon nitride tip with a tip radius of 50-70 nm and a peptide-coated surface was measured using an atomic force microscope (AFM). To determine the adhesive force between the AFM tip and the coated surface the AFM was used in "contact" mode. The tip was approached to the surface, allowed to make contact, and the deflection of the cantilever when the tip is pulled away from the surface was measured. The total amount of deflection, which reflects the adhesive force, was detected by laser beam. The adhesive force can be determined quantitatively from the beam deflection if the spring constant of the cantilever is known. In this study, the cantilever spring constant was 0.06 N/m. For each experiment, between 20 and 50 adhesive force measurements were obtained per sample.

The results of the adhesion study with stainless steel samples coated with either K122-4 or PAO pilin peptide are plotted in FIGS. 2A and 2B, respectively. As seen in FIG. 2A, the adhesive force for the coated metal clustered in a range between about 5-40 nN (nanoNewtons), with an

average around 20 nN, compared with an uncoated sample, where the adhesive force clustered between about 40-75 nN, with an average around 60 nN. Similar results were obtained with the PAO pilin coating. Since adhesive force is a reflection of electron activity, e.g., Van de Walls interactions, it can be concluded that the peptide coating serves to mask the metal surface electron layer.

Similar adhesion measurements on peptide-coated aluminum plates showed virtually no difference in the adhesion force between coated and uncoated plates.

Work Function Measurements

The electron work function (EWF) of coated and uncoated stainless steel samples was measured conventionally with a SKP370 Scanning Kelvin Probe. The technique operates using a vibrating capacitance probe, and through a swept backing potential, the work function difference is measured between the scanning probe reference tip and sample surface. The samples investigated were like those used in the adhesion studies, except that a sample coated with PAK pilin peptide was also examined.

The results of the study are plotted in FIG. 3A for uncoated and K122-4 pilin peptide coated samples, and in FIG. 3B, for uncoated and PAO- and PAK pilin peptide coated samples. For all three coating, the pilin peptide coating elevated the surface EWF by at least about 0.5 eV, to a final value of about 5 eV.

Similar EWF measurements on peptide-coated aluminum plates showed virtually no difference in the EWF between coated and uncoated plates.

Stability of the Peptide Coating

FIG. 4 shows the results of measurements on the K122-4 pilin peptide-coated sample taken over a 2-month time period after coating. The greater EFW of the coated sample with respect to the uncoated slide was observed over the two-month study period, indicating a coating stability of at least two months.

Nanoindentation/Hardness

A triboscope (Hysitron, Minneapolis, USA) was used to examine the changes in the mechanical properties of peptide-coated samples. The triboscope is a combination of a nanomechanical probe and an AFM. The probe, a diamond pyramidal Vickers indenter, has a nominal radius of 150 nm with a force sensitivity of 100 nN and a displacement resolution of 0.2 nm. During nanoindentation, a force-depth curve is obtained for each indentation and the total depth displacement of the tip into the surface of the sample was obtained from this curve. Nanoindentation tests were performed using forces of 50 to 800 μ N. Five force-depth curves were obtained for each force load.

The force-displacement curves of peptide-coated (dark) and uncoated (light) stainless steel under a range of loads between 50 and 800 μ N are shown in FIG. 5. Total displacement at the 800 μ N load is shown at the top of the graph, and is in the range 45-55 nm for the coated slide and is between about 90-95 nm for the uncoated slide. Based on this test, the coated slide has nearly twice the hardness as the uncoated slide. More generally, the coating is effective to increase the hardness of a stainless steel, tin, iron, or titanium metal surface by at least about 20%, preferably at least about 30%, and up to 50% or more.

FIGS. 6A and 6B plot displacements with nanoindentations produced at 20 μ N (6A), and 50 μ N (6B) for coated and uncoated slides. Consistent with the data from FIG. 5, coated slides had a hardness of between about 20%-100% greater than the uncoated slides.

The same type of study is plotted in FIGS. 7A and 7B for PAO-coated (7A) and K122-4 coated (7B) over a force range

of 50-800 μ N (7A) and 50-400 μ N (7B), with substantially the same results. In both cases, the pilin peptide coated nearly doubled the surface hardness of the metal sample.

Similar nanoindentation tests on peptide-coated aluminum plates showed virtually no change in surface hardness between coated uncoated plates.

Increased Conductance

Conductance is a measure of the ability of a material to conduct current flow. One standard method for measuring surface conductance uses an atomic force microscope (AFM) to measure the electrical current that flows from a specific location on the surface to the AFM tip under a specified low-voltage potential bias. The AFM displays the current flow (in pA) between the surface and the tip quantitatively as a specific color, represented by different shades of gray in FIGS. 8A and 8B for uncoated and pilin-coated stainless steel plates, respectively. In general, darker to lighter shades indicate greater to smaller currents (between 28.0 to 24.5 pA). From the two figures it is observed that surface regions of the uncoated stainless steel sample in FIG. 8A are quite variable over the sample surface and has predominant dark shading indicating a relatively high current flow, whereas the surface regions in the pilin coated sample in FIG. 8B are predominantly low conductance and substantially more uniform in conductance profile. The results are consistent with the EWF data from FIGS. 3A and 3B, showing substantially higher metal work function (a measure of the work needed to extract a surface electron) in the pilin-coated material.

Corrosion Resistance

There are a number of techniques available for investigating corrosion resistance or susceptibility to corrosion in a material surface. One method is to measure the current across a metal plate at a fixed potential. The measured current reflects the surface electrons to shuttle between redox forms, with a higher current indicating a greater potential for corrosion. The plot in FIG. 9A shows the measured current (Icorr) for 304 2B finish plates (20 gauge) stainless steel sheets prepared as above, either uncoated or coated with K-122-4 pilin peptide. The results show a significantly lower Icorr for the coated plates, indicating greater corrosion resistance.

It can be asked whether the difference in Icorr observed in the above study is related to the potential (Ecorr) at which current first begins to flow across the metal surface. This question was investigated by looking at the potential (Ecorr) at which current in the metal first begins to flow. The results of the study, shown in FIG. 9B, indicate that both the coated and uncoated metal samples have similar Ecorr values, indicating that the differences in Icorr values seen in FIG. 9A are not due to differences in the voltage potential response between the two samples.

Corrosion rate measurements, measured in mills (milli inches)/year (mpy) for the K-122-4 coated and uncoated samples are plotted in FIG. 10A. The results are consistent with the differences in Icorr seen in FIG. 9A. In particular, the pilin peptide coating appears to reduce corrosion rate over threefold, when average rates are compared.

Another widely used technique in corrosion monitoring is polarization resistance, defined as the slope of the potential current density curve at the free corrosion potential, yielding a resistance value Rp that can be related to corrosion current by a known mathematical relationship. FIG. 10B plots the Rp values for coated and uncoated stainless steel values, showing that the peptide coating significantly enhances Rp as a measure of corrosion resistance.

Interestingly, the effect of the pilin peptide in inhibiting corrosion can be reversed when the pilin is conjugated to another peptide having a strong dipole and/or high charge density, in this case, a leucine-zipper type E coil, or the same E coil having bound thereto, an oppositely charged K coil in an E coil/K coil pair. As seen in FIG. 11, the corrosion rate for the uncoated stainless steel sample is substantially lower than that sample having bound pilin in a pilin-E or E/K coil form.

The visual effect of corrosion testing on the various stainless steel samples discussed above is seen in FIGS. 12A-12C. In this study, samples were either uncoated (12B) or coated with a pilin peptide (12A) or a pilin peptide conjugated to an E/K coiled coil pair. In each case, the sample was subjected to previously described corrosion testing in a weak salt solution. The pilin-coated plate shows very little surface corrosion compared with the uncoated plate, while the pilin-conjugate coating appears to significantly enhance corrosion.

In summary, coating a metal such as stainless steel, tin, iron, or titanium with a synthetic pilin peptide containing a disulfide loop derived from the C-terminal receptor binding protein of Type IV *P. aeruginosa* (T4P) pilin and containing 0-10, preferably 0-5 additional residues on either or both the N- or C-terminal side of the loop, is effective to increase both the hardness and corrosion resistance of the metal surface. The increased corrosion resistance is evidenced by an change, e.g., increase in the electron work function of the metal surface by at least 0.2 eV, as well as the i_{corr} , corrosion rate, and R_p values described above. The increased hardness is evidenced by the reduced nanoindentation produced striking the metal surface with the tip of an atomic force microscope with a given force by at least 20%. [0120] Other metals contemplated in the method are transition metals from rows 4-6 and columns 9-12 of the periodic table, including cobalt, nickel, copper, zinc, ruthenium, rhodium, palladium, silver, cadmium, osmium, platinum, gold, and mercury, and mixtures and alloys thereof, and the metalloids silicon and germanium, and oxides thereof.

Reduced Coefficient of Friction

Coefficient of friction is measured using an atomic force microscope (AFM) according to known methods, e.g., as reported in 2007 J. Phys.: Conf Ser 61 51. Briefly, the cantilever beam of an AFM is applied to the surface with a given force, e.g., 500 mN, and moved across the test surface. The amount of deflection in the beam then provides a measure of the frictional force experienced by the beam, with a lower frictional coefficient producing a lower deflection. The deflection profile across the line of beam travel on the surface then provides a measure of frictional coefficient, with a small and more uniform deflection indicating a low coefficient, and a higher and more erratic profile, a higher beam deflection.

IIIB. Additional Evidence for Covalent Bonding of the Pilin Peptide to the Treated Metal

The altered electron work function of a coated metal and the enhanced resistance to erosion indicate that the pilin peptide has altered the electronic properties of the coated surfaces, suggesting formation of a covalent bond between the peptide and metal that alters the free-electron orbitals of the metal. Additional support for this finding comes from the peptide displacement assay and X-ray photoelectron spectroscopy (XPS) study reported in this subsection.

Peptide/pilin displacement assay One indicator of covalent interaction between a compound and substrate is the inability of the compound to be displaced from the substrate when the complex is incubated in the presence of the compound in

solute form. Here, the ability of exogenous pilin peptide to displace pilin peptide bound to a stainless steel surface was investigated. Commercial grade 304 2B finish plates (20 gauge) stainless steel sheets 1 mm thick were cleaned as previously described. These sheets were not annealed or polished. The sheets were assembled into a 96-well Schleicher and Schuell Minifold TM System (Mandel Scientific). Fifty microliters of a solution containing 10 $\mu\text{g/mL}$ of biotinylated PAK peptide or biotinylated purified pili were added to wells (5 replicates) and the manifold was incubated at RT for 1 hour with gentle agitation. Wells were washed six times with 1xPBS. Unlabeled PAK peptide was added to replicate wells in increasing amounts (0 to 10 $\mu\text{g/mL}$) and the steel manifold was incubated 1 hour at RT with gentle agitation. Wells were subsequently washed six times with PBS. Displacement of the bound biotinylated peptide or pili was assessed using streptavidin-horseradish peroxidase (HRP). Streptavidin-HRP (Sigma) was diluted 1/500, 100 μL were added per well, and the manifold was incubated for 1 hour at RT. One hundred and fifty microliter of developing buffer (0.01 M sodium citrate buffer pH 4.2 containing 1 mM 2,2'-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) (Sigma) and 0.03% (v/v) hydrogen peroxide) were added per well. The manifolds were incubated at RT for 10 min with gentle agitation. The reaction solution was transferred to a 96 well flat-bottomed micro titer plate (Corning) and the absorbance at 405 nm was determined using a FLUOstar OPTIMA plate reader (BMG LABTECH).

As seen from the data plotted in FIG. 13, there was no measurable loss of bound pilin peptide from the metal surface, even at relatively high concentrations of soluble pilin peptide, demonstrating that the bound pilin peptide is in equilibrium with unbound peptide, indicating a covalent bond attachment between the peptide and metal surface. Further evidence of the covalent bonding of the peptide to the metal surface is provided by the corrosion resistance studies below.

XPS Characteristics

X-ray photoelectron spectroscopy (XPS) is a quantitative spectroscopic technique that measures the elemental composition and electronic state of the elements that exist within a material. XPS spectra are obtained by irradiating a material with a beam of X-rays while simultaneously measuring the kinetic energy and number of electrons that escape from the top 1 to 10 nm of the material being analyzed. XPS requires ultra high vacuum (UHV) conditions.

An Axis-165 Spectrometer (Kratos Analytical) was used to examine the photo-emitted electrons from an uncoated and pilin-coated samples. The spectra for emitted electrons from the two samples are shown in FIG. 14. As seen, the pilin-coated sample contains two unique peaks with binding energies around 100 and 150 eV that are not present in the uncoated sample. One possibility is that the two peaks represent sulfur-metal bonds that are substantially red shifted because of conjugated electron bonding. There is no evidence of N or O bonding with the metal, suggesting that the likely covalent (shared electron) bond interaction between the pilin and metal is with one or both of the two sulfur atoms in the pilin peptide.

IIIC. Grain Boundary Effects

A grain boundary is the interface between two grains, or crystallites, in a polycrystalline material. Grain boundaries are defects in the crystal structure, and tend to decrease the electrical and thermal conductivity of the material. The high interfacial energy and relatively weak bonding in most grain

boundaries often makes them preferred sites for the onset of corrosion and for the precipitation of new phases from the solid.

Since a grain boundary can serve as an initial site for corrosion, it was of interest to determine whether pilin peptide binding to a metal surface occurred preferentially at grain boundary sites. To investigate this question, the adhesive force studies described above using a pilin-peptide coated AFM tip were further refined to investigate adhesive force effects within a grain and at a grain boundary. The “test” and “control” surfaces in the study were stainless steel plates coated with a PAO pilin peptide or a peptide having a scrambled sequence of the PAO amino acids. For each sample, the adhesive force within a grain and at a grain boundary was measured.

As seen from the results given in Table 1 below, the pilin peptide had an adhesive force about 20 nN lower than the same material with the scrambled sequence within a grain boundary, and an adhesive force about 43 nN lower at a grain boundary. The results indicate either that the pilin peptide is localizing preferentially at the grain boundary, i.e., the peptide has a greater coating thickness at the grain boundary, or that the same level of pilin binding produces a greater adhesive force effect at a grain boundary. In either case, the data may explain the magnitude of the anti-corrosion effect seen by binding a pilin peptide to a metal surface.

TABLE 1

Grain boundary region contributions to adhesive force.				
AFM Tip	Competitor	Adhesive Force Within Grain (nN)	Adhesive Force at Grain Boundary (nN)	Fold Increase at Grain Boundary
Coiled-coil-PAK (128-144)ox	PAO(128-144)ox	39.5 ± 9.4	44.5 ± 11.6	1.13
Coiled-coil-PAK (128-144)ox	PAO(128-144)ox_Scrambled	59.7 ± 8.4	87.5 ± 14.2	1.47
Attributable to PAK(128-144)ox		20.2 ± 17.9	43.0 ± 25.8	2.12

IIID. Pilin Peptide Binding by D- and RI-Forms of Pilin

To examine the ability of D- and RI-forms of pilin peptide to bind to a variety of materials, including various metals including stainless steel and polymers, and the characteristics of the pilin-coated materials, D- and RI forms of the K122-4 pilin peptide were synthesized and tested against the L form of the same pilin peptide.

In one study, stainless steel plates were coated with either the L-form pilin peptide (three different batches), the D-form pilin, or RI-form pilin, and one plate was uncoated. The plates were first examined for changes in adhesive force, similar to the study reported above with respect to FIGS. 2A and 2B. As seen in FIG. 15A, substantial reductions in adhesive force were seen for all three forms of the pilin peptide relative to the uncoated plate.

EWf measurements on the same six samples, conducted as described above with respect to FIGS. 3A-3B, are plotted in FIG. 15B. Interestingly, the D-amino acid form gave a substantially lower EWf than any of the other five plates, including the uncoated plate, whereas the RI pilin peptide significantly increased the EWf of steel beyond that observed for the stainless steel having bound L-form pilin peptide. These results are also seen in FIG. 27A which shows EWf values measured for stainless steel (Borg, Grade

304 stainless steel) coated with the L-form K122-4 pilin peptide (designated borg-K122SS), or coated with the D-form K122-4 pilin peptide (designated borg-K122SS-D-amino), or coated with the retro-inverso K122-4 pilin peptide (designated borg-K122SS retro-inverso). The retro-inverso pilin peptide was, as anticipated, resistant to trypsinization (both FIG. 27B) and also significantly increased the hardness of steel (FIG. 27C). As EWf is a measure of the energy level of the surface electrons and the ability of those electrons to react with other materials, an elevated EWf generally reflects enhanced resistance to corrosion. This is confirmed by the study shown in FIG. 27D, demonstrating that the borg-K122SS retro-inverso has a corrosion resistance rate about 50% that of uncoated stainless steel.

The results indicate that both D- and RI-forms of a pilin peptide are able to interact with a stainless steel plate in a manner that alters the electronic properties of the metal surface, with the retro-inverso form giving a substantially higher EWf than that of the L-form pilin peptide, and a much higher EWf than with the D-form pilin peptide.

The ability of the D-form pilin to bind tightly to stainless steel was investigated by comparing peptide binding to a stainless steel stent that was either uncoated, coated with a biotinylated control peptide (either a scrambled or a non-binding regions of a pilin sequence), or coated with a biotinylated D-form pilin peptide. The amount of peptide bound to each stent surface was measured by first washing the surface with a 3% solution of SDS at 37° C., followed by several more washes in PBS. The washed surfaces were incubated with streptavidin-HRP (horse radish peroxidase), then exposed to an ABTS substrate, and the absorbance read at 405 nm. As seen in FIG. 16A, about twice as much D-form pilin peptide bound to the stainless steel stent as did the control peptide.

To investigate the resistance to enzyme proteolysis of a metal-bound D-form pilin peptide, relative to a metal-bound L-form pilin peptide, stainless steel plates were coated in duplicate with the L-form peptide, D-form peptide, and control (scrambled pilin sequence). One sample from each duplicate was then incubated with trypsin, at a concentration of 0.25%, EDTA 1 mM, pH 7.4 at 37° C. for 60 minutes. Thereafter the samples were assayed for bound protein by the HRT assay method described above.

FIG. 17A shows bound peptide levels for the L-form pilin before and after exposure to trypsin. As seen, more than half the pilin peptide was removed by the protease treatment. By contrast, the amount of bound D-form pilin peptide was substantially unaffected by protease digestion (FIG. 17B). The results demonstrate that (i) the covalent binding of an L-form peptide to stainless steel does not confer protection against protease digestion, and (ii) the bound D-form pilin peptide is substantially protected against enzyme proteolysis.

Additional studies conducted in support of the invention demonstrate the significant advantages, in terms of higher binding affinity and resistance to proteolysis provided by the D-form and retro-inverso pilin peptide over the L-form pilin peptide. These advantages are particularly striking for the D-form pilin peptide, as will now be seen.

FIGS. 28A-28C show the effects of various D and RI forms of pilin peptides and peptide-PEG conjugates on unpolished (FIG. 28A) and titanium surfaces polished with 600 grit (FIGS. 28B and 28C). As seen, both D- and RI-forms of the K122 pilin peptide measurably increased the metal surface EWf, with the D-form giving the greatest EWf increase on both titanium surfaces. The PEG-D-pilin

peptide (FIG. 28C) enhanced EWF, but to a lesser degree than the non-pegylated D-form peptide.

Nanoindentation measurements a titanium surface coated with various D- and RI-form pilin peptides, including a PEG-D-pilin peptide are shown in FIG. 29. Both RI and D-forms of the peptide, including the PEG conjugate D-form peptide all showed a significant surface hardening over uncoated titanium. The PAO-K1301 peptide is an L-form pilin peptide having an amino acid mutation at residue position 130 (according to the number scheme of the Pi1A protein), where the native lysine residue is replaced by an isoleucine residue.

To demonstrate that the surface hardness effect seen in FIG. 29 is confined to the surface, e.g., not a bulk-metal effect, microindentation measurements were made on a steel surface coated with the same pilin peptides as in FIG. 29, but at the higher striking forces indicated in FIGS. 30A-30E. As seen, no change in hardness is seen at the higher forces.

FIG. 31A show the effect of PEG-D-pilin peptide coating on adhesive force on a titanium plate (FIG. 31A) and corrosion rate of a steel plate (FIG. 31B). The pegylated D-form peptide had little effect on surface adhesion, but significantly reduced the corrosion rate of a steel plate.

To demonstrate that a pegylated pilin peptide significantly reduces the frictional coefficient of a coated metal, in this case, a titanium plate, AFM frictional coefficient measurements on an uncoated titanium surface, and titanium coated with various pilin peptides were performed, as described above, with the results shown in FIGS. 32A-32E. Each figure is a representative profile taken from among six different profiles generated for each coating. The uncoated titanium surface gives high and erratic displacements as seen in the FIG. 32A profile. The lowest frictional coefficient was observed in the titanium plate coated with the PEG-D-peptide, shown in the FIG. 32B profile. The profile is characterized by low and quite uniform displacements over the surface line investigated. Although the non-pegylated RI-form of the peptide (FIG. 32C) and D-form (32D) significantly reduced the frictional coefficient, neither profile shows the reduction in frictional coefficient comparable to the PEG-D-peptide. Some improvement was observed when both D and RI forms of the non-pegylated peptides were applied (FIG. 32E), indicating that the D- and RI-forms are binding to different sites on the metal surface. However, the combined non-pegylated peptides still gave higher and more erratic displacements than the PEG-D-peptide alone.

Frames 2-9 in FIG. 33A are fluorescent micrographs of a stainless steel stent (shown in 20x in frame 1) whose surface has been coated with a control peptide (frames 2 and 3), an L-peptide (frames 4 and 5), an RI pilin peptide (frames 6 and 7) and a D-form pilin peptide (frames 8 and 9). As seen, the D-peptide retained the strongest fluorescence after incubation with whole blood, indicating the greatest resistance to proteolysis. FIG. 33B shows peptide/protein spots detected using streptavidin-horseradish peroxidase (linked to biotinylated peptides/proteins). On stainless steel, only the D-form and RI pilin peptides are resistant to proteinase K treatment. The two pilin peptides may also bind more effectively to the stainless steel substrate than the L-form pilin peptide. Similar results were seen when L-form, D-form and RI pilin peptides were bound to titanium (33C), where right panel A shows that the D-form peptide has greatest proteinase K resistance when bound to a titanium substrate. Similar results were observed for the binding of D-form and RI pilin peptides to a polyurethane substrate, as seen in FIG. 33D, which shows levels of binding of D-form and RI pilin peptides to the substrate, relative to a control

peptide. Although not shown here, the L-form peptide showed relatively weak binding to polyurethane

Similar results were obtained when the three pilin peptide forms were bound to a polysulfone dialysis membrane and then exposed to various disruptive treatments. Looking at frame A or FIG. 33E, it is seen that both the D-form and RI pilin peptides were substantially more resistant to trypsin digestion than the L-form peptide. Frame B of FIG. 33E shows the relative amounts of bound peptides after treatment with SDS, proteinase K, and boiling for ten minutes. In each case, the D-form peptide was the most stable when bound to the membrane, the L-form the least stable, with the RI peptide showing intermediate stability. This pattern was also seen for the three peptides bound to a silicone substrate (FIG. 33F).

It was of interest to investigate the relative stability of the three pilin peptide forms when the stainless steel substrate to which they were bound was subject to a mild electrical current. In one study, whose results are shown in FIG. 34, application of an 18V, 50 mA current across the stainless steel substrate for 10 minutes resulted in a virtual depletion of control peptide, the L-form peptide and the RI peptide, but with the D-form peptide showing little or no loss of bound peptide. Similar results were achieved at lower currents (30 and 40 mA over the same time period (data not shown). These results are particularly pertinent to various assay formats, described below, in which a ligand-binding receptor molecule is bound to a metal substrate through a pilin peptide. The ability of the D-form pilin peptide to retain its binding when the substrate is exposed to a mild current allows the substrate to be cleared of non-specifically bound material, by application of a moderate current across the substrate, without significant loss of signal relating to binding to analyte to the receptor, which is anchored to the substrate through a D-form pilin peptide.

IIIE. Related Applications

The ability of pilin peptide binding to increase the hardness of the coated material can also be exploited, in accordance with another aspect of the invention, to surface harden other materials, such as plate glass or automobile safety glass. In this application, a cleaned glass surface is contacted with the pilin peptide under conditions effective to coat the surface with a layer of the pilin. The amount of protein bound to the substrate was measured as above, using an HRP assay. As seen in FIG. 16B, the D-form peptide was tightly bound to the glass surface, resisting removal even by SDS treatment, and about twice as much pilin peptide bound to the stainless steel stent as did the control peptide.

In another application, the surface treatment is used to enhance the lubricity of coated metal surfaces that are in moving contact with one another in a machine. Here the target machine components are pretreated for enhanced surface lubricity by exposing the parts to a pilin peptide, as above, under conditions to form a covalently attached pilin coating. Alternatively, a solution of the pilin peptide may be applied to contacting surfaces of the machine during operation or during temporary shutdown, to maintain the greater lubricity of the machine components during machine operation.

IV. Coated Metal Substrate and a Biosensor Device

This section considers applications of the present invention to diagnostic devices in which an analyte-specific target compound, e.g., a receptor, is attached to a detection surface through a pilin peptide or peptide conjugate in accordance with the invention. Where the detection surface is a metal to

21

which the pilin peptide covalently bonds, through electronic interactions with the metal surface, the device may function in an electronic biosensor mode, as described below.

IVA. Metal Substrate with Covalently Bound Compound

This aspect of the invention includes a metal substrate to which a compound, e.g., receptor, is covalently attached at the substrate surface by means of (i) covalent attachment of a pilin peptide to the substrate, as detailed above, and direct or indirect covalent attachment of the compound to the pilin, i.e., through a pilin-compound conjugate, or direct attachment to a PEG moiety. The coated substrate is formed either by first attaching an unconjugated pilin peptide or conjugate to the metal surface, followed by covalently linking the compound to the bound pilin or PEG, or by first forming the pilin-compound conjugate or PEG-pilin-compound conjugate, followed by binding the conjugate to the metal surface, as described above for an unconjugated pilin peptide. Methods for covalently attaching a compound to the pilin peptide, e.g., by direct chemical coupling through amine or carboxyl groups, or using bifunctional coupling reagents, are well known. Where the compound is itself a peptide, the pilin-compound conjugate can be formed as a fusion protein, by recombinant or solid-phase synthesis. The coated substrate has altered surface electronic properties by virtue of the pilin binding to the metal surface, and studies conducted in support of the invention, detailed below, show that current flow across the substrate surface is modulated by binding of an analyte-related molecule to the surface-bound compound, making it possible to record such binding events by a change in current flow across the substrate. As will be seen below, the compound may also be covalently attached indirectly to the substrate, e.g., through an E/K coiled-coil complex. As can be appreciated from the data in FIGS. 33A-33F, the D-form of the pilin peptide is preferred in this application, given its more stable binding characteristics to a variety of substrate material.

IVB. Biosensor Device with Bound-Pilin Substrate

FIGS. 18A and 18B illustrate a biosensor assay device constructed in accordance with an embodiment of the invention. The device takes advantage of the altered electronic properties observed with a pilin-peptide coating on the metal detection plate 34. That is, plate 34 is formed of a metal such as stainless steel, tin, iron, or titanium, having altered electronic surface properties when coated by the pilin peptide. As above, the pilin coating is formed by exposing the plate surface with a conjugate of the above pilin peptide 36 and an analyte-binding moiety 38, or by attaching the compound to the bound, unconjugated pilin peptide. The plate itself forms the lower surface of a shallow biosensors reaction container 41 whose sides are formed by a wall 40 in the device, and which is partially filled with an aqueous conducting medium 42. The pilin peptide attached to the biosensor surface may include a combination of a PEG-pilin peptide conjugate, e.g., PEG-D-pilin, which functions to reduce nonspecific binding of sample material to the plate and a second peptide conjugate, e.g., R1-compound conjugate which functions to attach the compound covalently to the surface.

A biosensor circuit in the device includes an electrode 44 which extends into the container, a voltage source 46 and an ammeter 48, where the opposite circuit connection is to the lower side of the detection plate.

FIGS. 19 and 20 illustrate two surface configurations of the biosensor. In FIG. 19, a receptor (ligand-binding) molecules (R) is covalently attached to a pilin peptide (hook shaped moiety) which in turn is covalently coupled to the biosensor surface. Conductance across the biosensor sub-

22

strate is determined by (i) the bonding interaction of the pilin peptide with the substrate surface, and (ii) the effect of receptor R on conductance. When a ligand L, e.g., an analyte, binds to the receptor, the electronic properties at the surface are further modulated, causing a shift in observed biosensor current. The current shift indicated in FIG. 21 is from higher to lower current after ligand binding to the receptor.

In FIG. 20, the receptor R is coupled indirectly to the substrate surface through covalent bonding of a pilin/K coil conjugate to the substrate surface, followed by a coiled-coil interaction with a receptor-E coil conjugate. In this embodiment, the conjugate forming the biosensor surface coating is a three-component conjugate of the pilin peptide and a K coil, an E coil conjugated to the a receptor R and a ligand L which can bind to the R. In this embodiment, the biosensor is first reacted with analyte, to bind analyte to the coating, masking K-coil sites on the surface. This reaction may be read directly as a change in current caused by the masking of the K-coil, or an E-coil reagent may be added at this stage to bind to the E-coil in proportion to the amount of K-coil still unmasked after the analyte reaction.

FIGS. 22A and 22B are voltmeter cycle curves for a biosensor interaction in which a H is moiety (the receptor) is bound to the substrate surface through the coiled-coil configuration illustrated in FIG. 20. FIG. 22A shows the complete cyclic voltammetry curve for the H is moiety displayed on a K-coil complexed to an E-coil covalently linked to stainless steel via the pilin construct by itself and following the addition of an anti-His antibody specific for the H is moiety. FIG. 22B shows an amplification of the left hand portion cyclic voltammetry curve demonstrating that current flow is altered by antibody binding to the H is moiety not only under a positive voltage bias but also in a negative voltage bias. Binding of an anti-His antibody to the H is receptor produces the lowermost current cycle, evidencing a drop in current flow with "analyte" binding to the biosensor surface. Similar results were obtained when the H is receptor was bound directly to the biosensor surface through a pilin peptide, and the analyte employed was an anti-His antibody.

To understand the operation of operation of the sensor, it is useful to consider the corrosion resistance data from Table 2 below, which show I_{corr} , E_{corr} , Corrosion Rate, and R_p data for (i) an uncoated stainless steel plate (Unmodified) (ii) a stainless steel plate coated with a pilin peptide conjugated to an E-coil (negatively charged leucine zipper) peptide (E-PAK), and (iii) a third stainless steel plate coated with the same conjugate to which has been bound, the oppositely charged K-coil peptide, that is, the peptide is conjugated to an E-coil/K-coil heterodimer (K-E-PAK). Considering the I_{corr} column, the data show that E-PAK binding to the plate significantly increases its I_{corr} value, opposite the effect seen with the PAK pilin alone (see FIG. 9A). When the conjugate is neutralized (K-E-PAK), the I_{corr} value is now substantially less than the uncoated metal, similar to the effect observed for the unconjugated peptide. A similar effect on E_{corr} , corrosion rate, and R_p values is seen from the data, namely, neutralizing the E-coil effect by binding with the oppositely charged K coil significantly alters the surface effect produced by the E-PAK pilin binding.

TABLE 2

Corrosion resistance data for pilin conjugates on stainless steel				
Icorr (nAmps)	Ecorr (mV)	Corrosion Rate (mpy)	Rp (Ohms/cm ²)	
E-PAK	1940	87.2	0.1981	121.722
K-E-PAK	273	36.1	0.2492	130.145
Unmodified	802	4.2	0.0547	303.141

Various advantages of the biosensor can be appreciated from the above description. First, because the pilin peptide that covalently links the analyte-binding receptor to the biosensor surface directly affects the electronic active at the biosensor surface, altering the size and charge of the surface complex by ligand bind produces a direct effect, e.g., reduction, in current flow. Secondly, the interaction of molecules with metal surfaces is fundamentally different from the interaction with plastics, as electron activity or the ability of the surface electrons of the metal to interact directly with the molecule determines the degree and force of the interaction. Metals that do not form an oxide layer (such as gold) have very active surface electrons (due to edge effects of the crystals) and readily absorb materials to their surface. These materials are susceptible to the non-specific adsorption of proteins and other molecules from sample matrices and are therefore not useful as biosensor platforms. Metals such as stainless steel undergo surface oxidization to form a passive oxide layer (passivated), minimize non-specific binding events and do not readily bind materials to their surface (hence their widespread use in the medical and food industries). The ability to easily bind specific peptide/protein components to passivated stainless steel using the T4P17 peptide confers a significant advantage in improving the signal to noise ratio in detecting ligand-receptor interactions in biosensor applications. As stated above, T4P17 binding to stainless steel mediates electron transfer and can function as a biosensor when exposed to a voltage bias. The studies reported above demonstrate the ability to modulate electron flow across the biosensor surface in response to ligand binding to a pilin-receptor conjugate bound to the metal surface.

The R1 and D-form peptides offer competing advantages in the biosensor application. Because the R1 peptide produces a stronger electronic effect on the EWF, indicating a stronger delocalizing effect, the R1 peptide may provide an enhanced electronic response when an analyte or an analyte-related molecule binds to the receptor that is anchored to the substrate through the pilin peptide. On the other hand, the D-form peptide is likely to provide a more stable attachment of the receptor, and also allows removal of non-specifically bound sample material by applying a moderate current to the substrate, as discussed above. As noted above, the sensor surface may be coated with a combination of the two peptides, one or both having a PEG moiety.

IVE. General Assay Device with Bound-Pilin Substrate

FIGS. 23A and 23B show an analyte-detection device 16 having a plate 18 whose upper detection surface is coated with a conjugate of a pilin peptide 20 and an analyte-specific target molecule 22. Although the plate may be formed of a variety of materials to which the pilin peptide binds, it is preferably a metal, such as stainless steel, tin, iron, and titanium surface, to which the pilin binds covalently. The covalent binding provides a number of advantages in terms of ease of production, protein stability, coating stability. Also included in the device is a beam source 24, e.g., UV source, for irradiating the detection surface as shown at 25 and a

photo-detector 26. The peptide conjugate may additionally include a PEG moiety which can function to reduce non-specific binding to the surface.

In operation, the surface of the detection plate is covered with a fluid sample containing an analyte of interest, shown in FIG. 12B as soluble analyte molecules 30, and these are allowed to react with analyte-specific molecules 22 on the detection surface. In the fluorescence detector shown, the reaction mixture may contain a fluorescent-labeled antibody or other binding agent capable of reacting specifically with the analyte bound to the detection surface. The detection surface is then washed to remove unbound components. The reaction surface is now assayed for the presence and level of bound fluorescence, as indicated in FIG. 12B, showing emitted fluorescence at 27, to complete the assay.

It will be appreciated that convenient fluorescence assays may be incorporated into the device. For example, the pilin peptide may contain a fluorescent moiety and the analyte-binding moiety may include a fluorescent quencher effective to quench fluorescence from the pilin fluorescent moiety. In this embodiment, binding of analyte to the analyte-binding moiety is effective to mask the effect of the quencher, producing greater fluorescence in the presence of analyte binding to the analyte-binding moiety.

In an alternative embodiment, the pilin peptide and analyte-binding moieties may include first and second fluorescent species, respectively, that are effective to produce fluorescence resonance energy transfer, when excited at a given excitation wavelength. In this configuration binding of the analyte to the analyte-binding species is effective to inhibit such energy transfer, reducing the observed fluorescence.

As indicated above, the D-form peptide offers two significant advantages in this application: more stable binding to the substrate and the ability to remove non-specially bound material via an applied electrical current.

V. Medical Devices with Coated Metal Surfaces

The studies reported above on peptide binding to certain metal surfaces, e.g., stainless steel, tin, iron, and titanium surface, demonstrate that pilin peptide binding alters the surface electronic properties of the metal, indicating formation of a covalent (electron sharing) bond between the peptide and metal. This discovery provides a novel method for covalently attaching a bioactive molecule, e.g., peptide, lipid, nucleic acid, metabolite, or drug molecule, covalently to a stainless steel, tin, iron, and titanium surface, and novel medical devices having bioactive compounds covalently attached to an exposed device metal surface through a pilin peptide.

Other metals contemplated in the method are transition metals from rows 4-6 and columns 9-12 of the period table, including cobalt, nickel, copper, zinc, ruthenium, rhodium, palladium, silver, cadmium, osmium, platinum, gold, and mercury, and mixtures and alloys thereof, and the metalloids silicon and germanium, and oxides thereof.

In this method, the surface of the metal is contacted with a synthetic pilin peptide or PEG-pilin peptide conjugate containing a disulfide loop derived from the C-terminal receptor binding protein of Type IV *P. aeruginosa* (T4P) pilin and containing 0-10, preferably 0-5 additional residues on either or both the N- or C-terminal side of the loop.

If the pilin peptide or conjugate has been prepared in advance to include a covalently attached bioactive molecule, the contacting step alone results in the covalent attachment of the bioactive molecule to the metal surface. Methods for

25

preparing conjugates of peptides (in this case, a pilin peptide) with bioactive molecules are well known and include forming a fusion protein between the peptide and a peptide bioactive molecule, and the use of specific chemical modification reactions to provide reaction sites for covalent attachment of the bioactive molecule to the peptide. For example, the final step in the solid-phase synthesis of the pilin peptide may include the addition of a reactive group, e.g., aldehyde, that can be used for covalent reaction with the

Alternatively, the bioactive molecule may be reacted with the pilin peptide or PEG conjugate after peptide attachment to the metal surface, again employing conventional bifunctional reagents or specific chemical-group reaction chemistry to couple the bioactive molecule covalently to the bound pilin on the metal surface.

Another application, for use in producing and purifying a polypeptide of interest, is carried out by first synthesizing a fusion protein containing a pilin peptide of the type described above with the polypeptide of interest, e.g., by recombinant polypeptide synthesis. The fusion protein, which may be further modified by addition of a PEG group, is then contacted with a solid support formed of stainless steel, tin, iron, titanium, chromium, plastic, glass, silicate, ceramics, or a mixture thereof, thereby attaching the fusion protein to the support through attachment of the pilin peptide moiety to the support.

After washing the support to remove unbound material, the support is treated with an agent capable of specifically cleaving the polypeptide of interest from the bound pilin peptide. This may involve treating the support with a proteolytic enzyme capable of specifically cleaving a defined-sequence linker in the fusion protein, or treating the support with a chemical or a radiation energy source capable of specific cleavage of the linker in the fusion protein. The released polypeptide of interest is then eluted or washed from the support in substantially purified form.

Another application of the binding method is in preparing implantable devices that have desired surface properties or carry desired bioactive molecules on their surfaces. For example, a bone implant in accordance with the invention will include a stainless steel or titanium implant structure, a portion of which is adapted to be placed within or against a region of bone. To accelerate bond attachment to the implant, this portion is coated with a conjugate of a synthetic pilin peptide of PEG conjugate of the type described above and a bone-morphogenic factor, such as RGD or bone morphogenic factors BMP2-BMP7.

In a related application, the pilin peptide or PEG conjugate is applied to the surface of a metal or polymer stent, producing a stent in accordance with the invention that has improved surface properties, e.g., less tendency to promote surface reactions that can lead to undesired clotting or scarring at the intravascular implant site. The coating may alternatively be formed by a conjugate of a pilin peptide or PEG-pilin peptide conjugate and a bioactive molecule, such as pilin-limus drug conjugate having a bioreleasable linker, e.g., ester linker between the pilin and drug.

As will be seen below, the coated metal surfaces significantly reduce inflammatory response that the body can amount against the device.

VI. Medical Devices with Reduced Biofilm Formation and Reduced Inflammatory Response

In addition to inflammation mediated by infection or cellular injury/stress, a tremendous amount of iatrogenic

26

inflammation is induced by medical instrumentation. The exposure of human tissues, cells, and proteins to non-biocompatible medical devices triggers dysfunctional host responses, the clinical effects of which are greatly underestimated. Examples include tissue reaction and dysfunctional wound healing following the insertion of medical prostheses including vascular grafts, artificial joints and other implantable devices. Similarly, the activation of leukocytes and the coagulation system results in significant morbidity in critically ill patients regularly exposed to extracorporeal circuits such as cardiopulmonary bypass and hemodialysis. These events further impact healing, regeneration and rehabilitation of patients with acute and chronic diseases.

According to another aspect of the invention, it has been discovered that the inflammatory response to certain metals used in medical devices, e.g., titanium, by coating the metal surfaces with pilin peptides formed of D-amino acids, a mixture of D- and L-amino acids, and D-amino acids in a retro-inverso (RI) form, where all peptides forms may be further conjugated to PEG.

In one study, human peripheral blood mononuclear cells (PBMC) cells were incubated under standard cell-culture conditions either alone or in the presence of a titanium or steel plate, which was either uncoated or coated with the D-form pilin peptide. After 24 hours incubation in RPMI medium at 37° C., the culture medium was assayed for the cytokine IL-13, which is an indicator of an inflammatory response in the PBMC. Tubulin was assayed as a house-keeping control. FIG. 25A shows Western blots for IL-16 and tubulin measured for each of the five samples. As seen, the D-form pilin peptide coating was effective to inhibit the inflammatory response in both the stainless steel and titanium samples. This effect can be seen quantitatively for the titanium sample in the bar graph shown in FIG. 25B.

A similar study was conducted to test the inflammatory response of human THP-1 macrophage cells against the same samples, but including additional samples formed of stainless steel or titanium coated with control peptides 1 or 2 that represent non-binding pilin peptide sequences. The coated and uncoated samples were then incubated with THP-1 macrophages in RPMI medium at 37° C. for 72 hours. The culture media and cellular lysates were then assayed for the cytokine IL-13 and the housekeeping protein tubulin. The results are given in the two Western blots in FIG. 26A. As seen, titanium alone provoked a strong inflammatory response that was substantially diminished by the D-form pilin coating. The more quantitative picture for the stainless steel sample given in FIG. 26B shows that, although the inflammatory response to uncoated stainless steel was rather small, the effect was nonetheless inhibited by the D-peptide coating. It is apparent from the two control-peptide samples, which show a moderate and strong response, that the effect of D-form pilin coating is quite specific.

In one aspect, the invention includes a medical device having surfaces that are exposed to inflammatory-response cells when implanted in a body, in which these surfaces are coated with a synthetic pilin peptide containing (i) a disulfide loop derived from the C-terminal receptor binding protein of Type IV *P. aeruginosa* (T4P) pilin, (ii) 0-10, preferably 0-5, additional residues on either the N- or C-terminal side of the loop, and (iii) composed of D-amino acids, mixture of D- and L-amino acids, or D-amino acids in a retro-inverso (RI) form. The peptides may optionally be conjugated to a PEG moiety.

The binding studies in FIGS. 24A-24D demonstrate a strong bonding of a D-form pilin peptide to a variety of

medical devices and device surfaces, including stainless steel (FIG. 24A), a chromium cobalt stent (FIG. 24B), a latex catheter (FIG. 24C), and a silicone venous catheter (FIG. 24D). In each case, a sample was either uncoated (open bars in FIG. 24), coated with a control scrambled pilin sequence (shaded bars), or a D-form pilin peptide. The samples were then washed and assayed for bound protein by the HRT assay described above. For all samples, the levels of bound pilin peptide were substantially above control levels, with stainless steel appearing to form the most highly bonded coating. The invention includes other medical devices that are implanted or temporarily implanted in a subject body, including both metal and non-metal stents, catheters formed of a variety of flexible and materials, appliances supported at the end of catheters, and heart and other vascular valves, where the device includes surfaces exposed an inflammatory response in the body and is coated with a D-form or RI form pilin peptide, optionally conjugated to PEG.

In a related aspect, the invention also includes a method of inhibiting an inflammatory response against a medical device implanted in a subject by, prior to implanting the device, coating exposed surfaces of the device with a synthetic pilin peptide containing (i) a disulfide loop derived from the C-terminal receptor binding protein of Type IV *P. aeruginosa* (T4P) pilin, (ii) 0-10, preferably 0-5 additional residues on either or both the N- or C-terminal side of the loop, and (iii) composed of D-amino acids, mixture of D- and L-amino acids, or D-amino acids in a retro-inverso (RI) form, where the pilin peptides may optionally be conjugated to a PEG moiety.

In still another aspect, the invention includes a method of preventing or inhibiting biofilm formation by a non-*Pseudomonas* bacteria on the surface of an article by applying to the surface of the article, a D-form or retro-inverso synthetic pilin peptide containing a disulfide loop derived from the C-terminal receptor binding protein of Type IV *P. aeruginosa* (T4P) pilin and containing 0-10 additional residues on either or both the N- or C-terminal side of the loop, and optionally, a PEG moiety conjugated thereto, in an amount effective to inhibit biofilm formation by such non-*Pseudomonas* bacterium. The preferred pilin peptide is the D-form peptide, and a preferred peptide sequence contains the sequence identified as SEQ ID NO:10, as exemplified by the sequences identified as SEQ ID NOS:3, 4, or 9.

The article may be, for example, a non-medical device, such as a metal pipe, or polymeric dialysis membrane, that is subject to biofilm formation during normal use, or a medical device, such as a polymeric catheter, metal or polymer stent, heart valve or metal or ceramic bone prosthesis or bone implant. More generally, the coated surface in the article may be a metal, such as stainless steel, tin, iron, or titanium, a polymer, ceramic or silicate surface.

The method may be used, in specific examples, for inhibiting biofilm formation by a *Listeria* or *Staphylococcus* bacteria, where the amount of pilin peptide applied to the article surface is effective to inhibit biofilm formation by a *Listeria* or *Staphylococcus* bacteria, respectively.

In the studies reported below, the ability of the D-form or RI T4P pilin peptide to block bacterial binding of non-*Pseudomonas* bacteria to coated stainless steel plates was examined. Experimentally, stainless steel plates, either uncoated control or coated with D-form, RI or D-form+RI T4P pilin peptide (K-122-4) were incubated in a bacterial culture of 1×10^5 CFU/ml bacteria in 10 mM PBS pH 7.2 for 1 hour at room temperature with shaking. The plates were then washed 5 times, 5 ml/wash, with 10 mM PBS pH 7.2,

stained for 2 minutes at room temperature with 1 mM aqueous acridine orange, washed with water and then air dried. The plates were then examined by epifluorescence microscopy and 600 40 \times fields were photographed and the number of bound bacteria per field was determined. These conditions look at the initial stage of biofilm formation which is predictive of the ability to establish mature biofilms.

FIG. 35A shows the results for *Listeria innocua* binding to the plates, and demonstrates that both D-form and RI pilin peptide are effective in inhibiting binding of *Listeria innocua* to coated plates. For *Listeria monocytogenes*, strains H8, 19, and J10 (FIGS. 35B, 35C, and 35D, respectively), the D-form peptide provided significant protection against binding for all three strains, whereas the RI form provided a modest inhibitory binding effect only.

Similar results were obtained with various *Staphylococcus* species, as seen from the data in FIGS. 36A-36F, where the D-form peptide was generally, but always, the more inhibitory of the two pilin peptides.

Earlier studies with *Listeria monocytogenes* and *Staphylococcus aureus* failed to establish any difference in the bacterial binding to uncoated plates or plates coated with L-form pilin peptide, although it is known that L-form pilin peptide is effective in blocking binding of *Pseudomonas* bacteria to coated plates. Collectively, the results indicate that while the L-form T4P pilin peptide is effective in inhibiting biofilm formation by *Pseudomonas* bacteria, when coating a surface, inhibition of biofilm formation by non-*Pseudomonas* bacteria can also be achieved by the D-form and or RI T4P pilin peptide, with the D-form peptide being generally more inhibitory.

FIGS. 37A-37C show the effect of PEG-D-pilin peptide on biofilm formation on stainless steel surfaces. In each frame, the area coated with the PEG-D-peptide showed little or no biofilm formation, in contrast to the readily apparent biofilm growth in the uncoated regions of the metal surface. The inhibition applies both to a *Pseudomonas aeruginosa* bacterial strain (FIG. 37A) and to a *Staphylococcus aureus* bacterium. Studies conducted in support of the present invention indicate inhibition by the PEG-D-pilin peptide coating of a number of other bacterial pathogens. These observations are consistent with the published literature reports that indicate that modification of surfaces by the addition of PEG is effective in limiting biofilm formation, but where the limitation to the commercialization of the approach has been the fabrication of stable and long-lasting PEG surface treatments. The present invention solves that problem by attaching the PEG covalently to the metal surface through a pilin peptide, which itself has been shown by the inventors to inhibit biofilm formation on a peptide-coated surface.

VII. Coated Pipes and Methods

The ability of D- and RI-form pilin peptide conjugates of PEG to reduce frictional drag at a coated surface, as well as to increase hardness and reduce corrosion, has important applications in the drilling and pipe industry, for reducing frictional drag of fluids flowing through pipes, while enhancing hardness and corrosion resistance.

In oil and gas exploration, it is common to pump a liquid into bedrock formations under pressure, to fracture rock layers and release trapped natural gas, a process known as fracking. The fracking fluid is typically a slurry of water, proppants, such as sand, and chemical additives. By coating the interior surfaces of the pipe with a PEG-D or RI form

29

pilin peptide conjugate, the frictional drag of the slurry pumped into the pipe can be reduced significantly, reducing the cost and increasing the efficiency of the pumping operation. Similarly, for pipelines carrying oil or natural gas products over long distance, a significant improvement in transport cost and efficiency is achieved with the pipes having interior-surface coating.

In practicing the method of treating a pipe to reduce the frictional drag of fluid flowing through the pipe, a conjugate of (i) a D-form or retro-inverso synthetic pilin peptide containing a disulfide loop derived from the C-terminal receptor binding protein of Type IV *P. aeruginosa* (T4P) pilin and containing 0-10 additional residues on either or both the N- or C-terminal side of the loop and (ii) a polyethylene glycol moiety, is introduced into the pipe, in an amount effective to reduce the frictional drag of fluid flowing through the pipe by attachment of the pilin peptide to the walls of the pipe. The conjugate can be introduced into the pipe in a separate coating solution or suspension of the conjugate, or may be introduced by periodic addition of the conjugate to the fluid, e.g., fracking slurry, that is normally carried by the pipe. For example, conjugate can be periodically added to a fracking slurry to a final solution of 1-10 grams/kiloliters, for initial coating and periodic restoration of the coating. It will be appreciated that the sand particles in the slurry should be precoated with a PEG-pilin conjugates, as described below, to prevent the particles from binding to and removing a large portion of the added conjugate intended for coating the pipe interior surfaces.

A preferred pilin peptide is the D-form pilin peptide having a polyethylene glycol moiety covalently attached at one of both of the C- or N-termini of the peptide, where the polyethylene glycol moiety in the peptide conjugate has a molecular weight between 0.2 and 500 kDal.

The interior surface of the pipe to which the pilin peptide conjugate is attached may be a metal, polymer, ceramic or silicate surface. Exemplary metals include stainless steel, carbon steel, titanium, copper, brass, tin, iron, silver, and magnesium and alloys thereof. Where the pipe has a stainless steel interior surface, the amount of pilin peptide conjugate attached to the pipe's interior surface by the introducing is sufficient to reduce the corrosion of said interior surface.

A related aspect of the invention is an article having an exposed metal, polymer, ceramic or silicate surface or surfaces coated with a conjugate of (i) a D-form or retro-

30

inverso synthetic pilin peptide containing a disulfide loop derived from the C-terminal receptor binding protein of Type IV *P. aeruginosa* (T4P) pilin and containing 0-10 additional residues on either or both the N- or C-terminal side of the loop and (ii) a polyethylene glycol moiety, in an amount of conjugate sufficient to reduce the coefficient of friction of the exposed surface(s). The coated article has a reduced coefficient of friction, and in the case of metal articles, greater corrosion resistance. Exemplary pilin peptides, e.g., a D-form peptide, and PEG moieties are as described above.

The article is made by exposing the article surface(s) to be coated with a solution or suspension of the PEG-pilin conjugate, as described above for non-glycosylated pilin peptides coatings.

Where the article is a pipe whose interior wall surface is coated with the peptide conjugate, the peptide conjugate coating the surface is present in an amount sufficient to reduce the frictional drag of a fluid flowing through the pipe.

Where the articles is a microfluidics channel having a width dimension of 100 microns or less, the interior wall surface of the channel is coated with the peptide conjugate in an amount sufficient to reduce the frictional drag of a fluid flowing through the channel, for example, the frictional drag of a sand slurry through a drilling pipe.

In another embodiment, the moving parts of a machine, such as inter-digitating gears or reciprocating elements are coated with the peptide conjugate to reduce frictional drag and wear on the parts.

In still another embodiment, the article has surfaces, such as metal leads or electrodes, that are coated to protect the leads against corrosion or wear placed on the leads during initial assembly or replacement.

In another embodiment, the coated article is slurry particle, such as sand, which is coated by adding a PEG-D- or RI-form pilin conjugate to a suspension of the particles, or by passing a solution of the conjugate over a bed of the particles, or simply by adding the conjugate to the slurry. The reduced frictional drag of the particles, both with respect to particle-particle interactions, and particles interactions with the interior wall of a pipe, further reduces the flow resistance of the slurry through a pipe over that achieved by coating the interior of the pipe alone.

Although the invention has been described with respect to specific embodiments and applications, it will be appreciated with various modifications can be made without departing from the invention.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 21

<210> SEQ ID NO 1

<211> LENGTH: 39

<212> TYPE: PRT

<213> ORGANISM: *Pseudomonas aeruginosa*

<400> SEQUENCE: 1

Ser Ile Asp Trp Gly Cys Ala Ser Asp Ser Asn Ala Val Ser Ser Gly
1 5 10 15

Thr Asp Arg Asn Met Pro Ala Leu Thr Ala Gly Thr Leu Pro Ala Arg
20 25 30

Phe Ala Pro Ser Glu Cys Arg
35

-continued

```

<210> SEQ ID NO 2
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas aeruginosa

<400> SEQUENCE: 2

Thr Ile Asp Trp Ala Cys Thr Ser Ala Ser Asn Ala Thr Ala Thr Ala
1             5             10             15

Gln Gly Phe Thr Gly Met Ala Ala Gly Ser Val Pro Gln Glu Phe Ala
                20             25             30

Pro Ala Gln Cys Arg
              35

```

```

<210> SEQ ID NO 3
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas aeruginosa

<400> SEQUENCE: 3

Asp Gly Val Trp Ala Cys Lys Ser Thr Gln Asp Pro Met Phe Thr Pro
1             5             10             15

Lys Gly Cys Asp Asn
              20

```

```

<210> SEQ ID NO 4
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas aeruginosa

<400> SEQUENCE: 4

Asp Gly Leu Trp Lys Cys Thr Ser Asp Gln Asp Glu Gln Phe Ile Pro
1             5             10             15

Lys Gly Cys Ser Arg
              20

```

```

<210> SEQ ID NO 5
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas aeruginosa

<400> SEQUENCE: 5

Asp Gly Val Trp Asn Cys Lys Ile Thr Lys Thr Pro Thr Ala Trp Lys
1             5             10             15

Pro Asn Tyr Ala Pro Ala Asn Cys Pro Lys Ser
              20             25

```

```

<210> SEQ ID NO 6
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas aeruginosa

<400> SEQUENCE: 6

Glu Gly Val Trp Thr Cys Ala Thr Ser Gly Ser Pro Ala Asn Trp Lys
1             5             10             15

Ala Asn Tyr Ala Pro Ala Asn Cys Pro Lys Ser
              20             25

```

```

<210> SEQ ID NO 7
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas aeruginosa

<400> SEQUENCE: 7

```

-continued

Asn Gly Gly Trp Ser Cys Ala Thr Thr Val Asp Ala Lys Phe Arg Pro
 1 5 10 15

Asn Gly Cys Thr Asp
 20

<210> SEQ ID NO 8
 <211> LENGTH: 32
 <212> TYPE: PRT
 <213> ORGANISM: *Pseudomonas aeruginosa*

<400> SEQUENCE: 8

Gly Ser Ser Trp Ala Cys Gly Asn Ala Ser Ile Asp Gly Phe Ala Gly
 1 5 10 15

Thr Gly Thr Thr Ile Asp Ala Lys Tyr Leu Pro Asn Ala Cys Lys Pro
 20 25 30

<210> SEQ ID NO 9
 <211> LENGTH: 27
 <212> TYPE: PRT
 <213> ORGANISM: *Pseudomonas aeruginosa*

<400> SEQUENCE: 9

Ser Tyr Thr Trp Ala Cys Thr Ser Asn Ala Asp Asn Lys Tyr Leu Pro
 1 5 10 15

Lys Thr Cys Gln Thr Ala Thr Thr Thr Pro
 20 25

<210> SEQ ID NO 10
 <211> LENGTH: 17
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: synthetic consensus sequence
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (1)..(1)
 <223> OTHER INFORMATION: Xaa is Lys, Ala, Ser or Thr
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (3)..(3)
 <223> OTHER INFORMATION: Xaa is Thr, Lys or Ala
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (4)..(4)
 <223> OTHER INFORMATION: Xaa is Ser or Thr
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (5)..(5)
 <223> OTHER INFORMATION: Xaa is Asp, Thr or Asn
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (6)..(6)
 <223> OTHER INFORMATION: Xaa is Gln, Val or Ala
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (7)..(7)
 <223> OTHER INFORMATION: Xaa is Asp or Glu
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (8)..(8)
 <223> OTHER INFORMATION: Xaa is Glu, Pro, Ala or Asn
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (9)..(9)
 <223> OTHER INFORMATION: Xaa is Gln, Met or Lys
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (10)..(10)
 <223> OTHER INFORMATION: Xaa is Phe or Tyr
 <220> FEATURE:

-continued

```

<221> NAME/KEY: misc_feature
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: Xaa is Ile, Thr, Arg or Leu
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: Xaa is Lys or Asn
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (14)..(14)
<223> OTHER INFORMATION: Xaa is Gly or Thr
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (16)..(16)
<223> OTHER INFORMATION: Xaa is Ser, Asp, Thr, Gln or Asn
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Xaa is Lys, Asn, Asp or Thr

<400> SEQUENCE: 10

Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Pro Xaa Xaa Cys Xaa
1             5             10             15

Xaa

<210> SEQ ID NO 11
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas aeruginosa

<400> SEQUENCE: 11

Ser Tyr Thr Trp Ala Cys Thr Ser Asn Ala Asp Asn Lys Tyr Leu Pro
1             5             10             15

Lys Thr Cys Gln Thr Ala Thr Thr Thr Thr Pro
20             25

<210> SEQ ID NO 12
<211> LENGTH: 33
<212> TYPE: PRT
<213> ORGANISM: Ralsonia metallidurans

<400> SEQUENCE: 12

Ser Val Thr Trp Gln Cys Glu Ser Ser Ala Asp Lys Arg Tyr Val Pro
1             5             10             15

Gln Ala Cys Ala Lys Ala Ser Glu Ser Gly Lys Thr Thr Thr Thr Thr
20             25             30

Thr

<210> SEQ ID NO 13
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Neisseria cinera

<400> SEQUENCE: 13

Ser Phe Ser Trp Val Cys Lys Lys Gly Thr Ser Asp Ser Val Asp Asp
1             5             10             15

Lys Phe Leu Pro Ser Ser Cys Arg Thr Ala Ala Thr Thr Thr Ala Gly
20             25             30

<210> SEQ ID NO 14
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Eikenella corrodens

<400> SEQUENCE: 14

```

-continued

Ser Phe Ser Trp Glu Cys Ser Ser Asn Ala Asp Ala Lys Tyr Leu Pro
1 5 10 15

Ser Ser Cys Arg Asn Ala Ala Thr Pro Thr Pro Thr
20 25

<210> SEQ ID NO 15
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas aeruginosa

<400> SEQUENCE: 15

Gly Ser Ser Trp Ala Cys Gly Asn Ala Ser Ile Asp Gly Phe Ala Gly
1 5 10 15

Thr Gly Thr Thr Ile Asp Ala Lys Tyr Leu Pro Asn Ala Cys Lys Pro
20 25 30

<210> SEQ ID NO 16
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Eikenella corrodens

<400> SEQUENCE: 16

Ser Phe Ser Trp Glu Cys Ser Ser Asn Ala Asp Ala Lys Tyr Leu Pro
1 5 10 15

Ser Ser Cys Arg Asn Ala Ala Thr Pro Thr Pro Thr Pro
20 25

<210> SEQ ID NO 17
<211> LENGTH: 46
<212> TYPE: PRT
<213> ORGANISM: Neisseria gonorrhoeae

<400> SEQUENCE: 17

Ser Val Lys Trp Phe Cys Gly Gln Pro Val Thr Arg Thr Gly Asp Asn
1 5 10 15

Asp Asp Thr Val Ala Asp Ala Lys Asp Gly Lys Glu Ile Asp Thr Lys
20 25 30

His Leu Pro Ser Thr Cys Arg Asp Thr Ser Ser Ala Gly Lys
35 40 45

<210> SEQ ID NO 18
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Xanthomonas cmpestris

<400> SEQUENCE: 18

Ser Ile Ser Trp Gly Cys Thr Asn Gly Thr Thr Ile Asp Gln Lys Tyr
1 5 10 15

Leu Pro Thr Ser Cys Arg Thr Ala Ala Ala Pro
20 25

<210> SEQ ID NO 19
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas aeruginosa

<400> SEQUENCE: 19

Asn Gly Gly Trp Ser Cys Ala Thr Thr Val Asp Ala Lys Phe Arg Pro
1 5 10 15

Asn Gly Cys Thr Asp
20

-continued

```

<210> SEQ ID NO 20
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Dichelobacter nodosus

```

```

<400> SEQUENCE: 20

```

```

Ser Gly Ser Trp Tyr Cys His Ser Asn Ala Ala Glu Lys Phe Leu Pro
1          5          10          15

```

```

Ser Gly Cys Lys Tyr Asp Ala Ser Leu
          20          25

```

```

<210> SEQ ID NO 21
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic consensus sequence
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa is Ser or Thr
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa is Gly or Ala
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa is Ala or Thr
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa is Asp or Ala
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: Xaa is Val or Thr
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (14)..(14)
<223> OTHER INFORMATION: Xaa is Ser or may be absent
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa is Ser or may be absent
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (16)..(16)
<223> OTHER INFORMATION: Xaa is Gly or Ala
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (18)..(18)
<223> OTHER INFORMATION: Xaa is Asp or Ala
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (19)..(19)
<223> OTHER INFORMATION: Xaa is Arg or Gln
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Xaa is Asn or Gly
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa is Met or Phe
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Xaa is Pro or Thr
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (23)..(23)
<223> OTHER INFORMATION: Xaa is Ala or Gly
<220> FEATURE:
<221> NAME/KEY: misc_feature

```

-continued

```

<222> LOCATION: (24)..(24)
<223> OTHER INFORMATION: Xaa is Leu or Met
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (25)..(25)
<223> OTHER INFORMATION: Xaa is Thr or Ala
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (28)..(28)
<223> OTHER INFORMATION: Xaa is Thr or Ser
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (29)..(29)
<223> OTHER INFORMATION: Xaa is Leu or Val
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (31)..(31)
<223> OTHER INFORMATION: Xaa is Ala or Gln
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (32)..(32)
<223> OTHER INFORMATION: Xaa is Arg or Glu
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (36)..(36)
<223> OTHER INFORMATION: Xaa is Ser or Ala
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (37)..(37)
<223> OTHER INFORMATION: Xaa is Glu or Gln

<400> SEQUENCE: 21

Xaa Ile Asp Trp Xaa Cys Xaa Ser Xaa Ser Asn Ala Xaa Xaa Xaa Xaa
1          5          10          15

Thr Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ala Gly Xaa Xaa Pro Xaa Xaa
20          25          30

Phe Ala Pro Xaa Xaa Cys Arg
35

```

It is claimed:

1. A method of treating a pipe to reduce the frictional drag of fluid flowing through the pipe, comprising

introducing into the pipe, a liquid carrying a conjugate of (i) a D-form or retro-inverso synthetic pilin peptide containing a disulfide loop of a C-terminal receptor binding protein of Type IV *P. aeruginosa* (T4P) pilin and containing 0-10 additional residues on either or both the N- or C-terminal side of the loop and (ii) a polyethylene glycol moiety, in an amount of conjugate effective to reduce the frictional drag of fluid flowing through the pipe by attachment of the pilin peptide to the walls of the pipe.

2. The method of claim 1, wherein the pilin peptide conjugate is a D-form pilin peptide having a polyethylene glycol moiety covalently attached at one or both of the C- or N-termini of the peptide.

3. The method of claim 1 or 2, wherein the polyethylene glycol moiety in the peptide conjugate has a molecular weight between 0.2 and 500 kDal.

4. The method of claim 1, wherein the pilin peptide in the conjugate introduced into the pipe contains the sequence identified as SEQ ID NO:10.

5. The method of claim 4, wherein the pilin peptide in the conjugate introduced into the pipe contains the sequence identified as SEQ ID NOS:3, 4, or 9.

6. The method of claim 1, wherein the interior surface of the pipe to which the pilin peptide conjugate is attached is selected from a metal, polymer, ceramic or silicate surface.

7. The method of claim 1, wherein the interior surface of the pipe to which pilin peptide is applied is a metal surface selected from the group consisting of stainless steel, carbon steel, titanium, copper, brass, tin, iron, silver, and magnesium and alloys thereof.

8. The method of claim 1, wherein said pipe has a stainless steel interior surface and the amount of pilin peptide conjugate attached to the pipe's interior surface by said introducing is sufficient to reduce the corrosion of said interior surface.

9. The method of claim 1, wherein the amount of pilin peptide conjugate attached to pipe's interior wall by said introducing is sufficient to reduce the frictional drag of a sand-containing slurry introduced into the pipe.

10. The method of claim 1, wherein said introducing includes periodically adding the peptide conjugate to fluid introduced into the pipe during normal operation.

11. In a method of treating a metal material having surfaces with exposed grain boundary regions, to reduce the rate of corrosion of the material, an improvement comprising

contacting exposed surfaces boundary regions in the material with a D-form or retro-inverso synthetic pilin peptide containing a disulfide loop of a C-terminal receptor binding protein of Type IV *P. aeruginosa* (T4P) pilin and containing 0-10 additional residues on either or both the N- or C-terminal side of the loop, and optionally, a polyethylene glycol moiety covalently attached to the peptide, in an amount effective to inhibit the rate of corrosion of the material.

12. The improvement of claim 11, wherein the pilin peptide conjugate is a D-form pilin peptide having a polyethylene glycol moiety covalently attached at one or both of the C- or N-termini of the peptide.

13. The improvement of claim 11, wherein the polyethylene glycol moiety attached to the peptide has a molecular weight between 0.2 and 500 kDal. 5

14. The improvement of claim 11, wherein the pilin peptide contains the sequence identified as SEQ ID NO:10.

15. The improvement of claim 11, wherein the pilin peptide in the conjugate introduced into the pipe contains the sequence identified as SEQ ID NOS: 3, 4, or 9. 10

16. The improvement of claim 11, wherein said contacting is effective to change the electron work function of exposed grain-boundary regions by at least 0.3 eV EFW units and to increase the hardness of exposed grain-boundary regions, as measured by nano-indentation produced striking the metal surface with the tip of an atomic force microscope with a given force, by at least 20%. 15

17. The improvement of claim 11, wherein the metal is selected from the group consisting of stainless steel, carbon steel, titanium, copper, brass, tin, iron, silver, and magnesium and alloys thereof. 20

18. The improvement of claim 11, wherein the metal material is stainless steel, and the contacting step is reduce the rate of corrosion of the coated surface, as measured by the corrosion current across the surface by at least 30%. 25

19. The improvement of claim 11, wherein the metal material is porous or reticulated, and the contacting step is be carried out to bind the pilin peptide to internal surfaces defined by pores or reticulations in the material. 30

* * * * *